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INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY  
RESEARCH LABORATORY

Gainesville, Florida

SECOND SEMI-ANNUAL REPORT - 1973



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SECOND SEMI-ANNUAL REPORT

1973

*Insect Attractants, Behavior, and Basic Biology*

*Research Laboratory*

*USDA-ARS, Southern Region, Florida-Antilles Area*

*P. O. Box 14565*

*Gainesville, Florida 32604*

In Cooperation with the Departments of

Entomology and Nematology

and

Agricultural Engineering

University of Florida

This progress report includes tentative results of research not sufficiently complete to justify general release. Such findings, when adequately confirmed, will be released through established channels. Therefore, this report is not intended for publication and should not be referred to in literature citations.



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Juvenile Hormone Binding by Haemolymph  
Proteins in the Indian Meal Moth

S. M. Ferkovich, D. L. Silhacek, and R. R. Rutter

(Continuation of report 01 73(1-6))

Objectives: To understand the transport of juvenile hormone (JH) in the haemolymph from its site of synthesis to target tissues. Others showed that lipoproteins may act as hormone carriers; their isolation should facilitate investigations of substances (hormone analogs, etc.) that interfere with their capacity to carry or unload JH at its site of utilization. We, therefore, are studying the binding affinity of JH for select blood proteins and the significance of such proteins in hormone transport in the Indian meal moth, Plodia interpunctella.

Methods: Haemolymph from 4, 8, and 21 mg fifth-instar larvae was incubated with  $^3\text{H}$ -JH and fractionated on G-200 columns and the molecular weights of the resolved protein peaks estimated. The proteins were analyzed by disc gel electrophoresis. JH and its metabolites were analyzed by thin-layer chromatography.

Results: In the previous report (73(1-6)), we stated that two lipoproteins selectively bound  $^3\text{H}$ -JH in vitro. However, attempts to demonstrate the juvenilizing effects of the JH-lipoprotein complexes by using an in vitro bioassay system (in cooperation with H. Oberlander) were unsuccessful. A more extensive examination of the JH-protein interactions has revealed that a smaller molecular weight protein (25,000 M.W.), rather than a lipoprotein, is likely the carrier for JH. Lipoproteins with an estimated molecular weight of 203,000 also bind the hormone but only when excess JH is present. These results suggest that the lipoproteins function as a reservoir for JH in the haemolymph and that the lower molecular weight JH-binding protein may act as the actual carrier for the hormone.

Plans: A manuscript is at peer review. We will determine whether properties of the JH-binding protein satisfy the requirements for a JH-transport molecule. Specifically, we will apply a preparative procedure to purify and collect the JH-binding protein. Once an adequate quantity of the purified protein is obtained, we will determine certain characteristics of the protein (type, specificity, etc.) and test the effectiveness of JH-protein complex using an in vitro bioassay system.



Effect of Juvenile Hormone and Its Mimics on Mitochondrial  
Metabolism in the Indian Meal Moth,  
Plodia interpunctella

D. L. Silhacek

(Continuation of report 03 73(1-6))

Objective: In studies on the effects of hormones on intermediary metabolism of Indian meal moths, we found that in vitro juvenile hormone (JH) inhibits some mitochondrial oxidations while stimulating others. These results suggested that the JH effects on mitochondrial metabolism might provide valid criteria for evaluating potentially active JH-mimics. The purpose of the present experiments was to determine the feasibility of developing such a method.

Methods: Mitochondria were isolated from Indian meal moth larvae by differential centrifugation. Mitochondrial oxidative activities with succinate and pyruvate-malate as substrates were determined with a vibrating platinum electrode. Initial experiments were conducted with mitochondria isolated from larvae of different known ages. Subsequent experiments were conducted with mitochondria isolated from newly molted last-instar larvae.

Results: JH stimulation of succinate oxidation by isolated mitochondria appears to involve transport processes associated with the mitochondrial membrane system. Studies elucidating the mechanism of JH action in mitochondria are still in progress. In vivo studies reveal that ingested JH affects mitochondrial metabolism in a manner similar to the in vitro effects; however, JH treatment did not prevent the normal "aging effects" on mitochondrial metabolism which occur as the insect grows to maturity.

Experiments examining the specificity and effectiveness of JH mimics were continued. Considerable insight into the structural requirements of the JH molecule has been derived from this study. Acquisition and synthesis of specific chemicals are now essential for testing the conclusions that have been reached.

Plans: Studies will continue on (1) determining what molecular structural characteristics are needed for affecting mitochondrial metabolism, (2) elucidating the mechanism of JH-stimulated succinate metabolism, and (3) determining the role of JH-induced mitochondrial metabolic changes in influencing the metabolism in other subcellular compartments.



Influence of Juvenile Hormone on Reproductive  
Behavior of the Indian Meal Moth

H. Oberlander, L. L. Sower and D. L. Silhacek

(Continuation of report 04 73(1-6))

Objective: To determine whether sublethal juvenile hormone treatment of larvae the Indian meal moth, Plodia interpunctella (Hübner) affects the reproductive behavior of insects which emerge as "normal" adults.

Methods: Larvae of the Indian meal moth were reared on juvenile hormone (ENT 33972a)-treated diet for varying periods of time. We tested the mating frequency, calling behavior, pheromone content, and attractancy to pheromone of control and hormone-treated insects.

Results: Treatment of Plodia throughout larval life with concentrations of juvenile hormone ranging from 0.25 to 25.0 ppm substantially reduced mating frequency. For effective suppression of mating, continuous treatment during the fourth and fifth instars was required. Calling behavior, pheromone content of females, and male response to pheromone were not substantially affected by the hormone treatment, and hence were not responsible for the reduced mating. These results emphasize the need for a behavioral component of juvenile hormone bioassays.

Plans: Long term effects and mode of action of juvenile hormone on reproductive behavior will be explored.

Publications: See App. 2 In Press: Dutkowski, A. B. and H. Oberlander; and Benson, J. and H. Oberlander. NTE: Oberlander, H. and C. E. Leach; and Silhacek, D. L. and H. Oberlander.



Exposure of Stored-Product Coleoptera to  
Hormone-Treated Culture Medium

F. O. Marzke and W. G. Sercey

(Continuation of report 05 73(1-6))

Objective: To determine the effect on development of exposure of the cigarette beetle, (Lasioderma serricorne) or red flour beetle, (Tribolium castaneum) to culture medium treated with juvenile hormone-mimicking compounds.

Methods: Cigarette beetle and red flour beetle culture medium (flour, corn meal, and brewers yeast) was treated with juvenile hormone-mimicking compounds at 0.5-250 ppm or higher. Eggs, last-instar larvae or adults were placed in the treated medium and the subsequent development of the insects observed.

Results: In studies reported under 06 73(1-6) egg hatch was prevented by exposing mated cigarette beetle females to paper treated with Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$ . In order to prevent egg hatch if mated beetles were exposed to treated medium, a concentration of more than 5000 ppm was required.

Medium aged 18 months after treatment with Zoecon 515 at 10 ppm or higher was still effective in preventing adult emergence when last-instar larvae of the cigarette beetle were exposed to the treated diet.

Plans: Preliminary studies indicated exposure of larvae to JH compounds may result in reduced adult fertility. These studies will be expanded.



Exposure of Stored-Product Coleoptera to  
Hormone-Treated Papers

F. O. Marzke and W. G. Sercey

(Continuation of report 06 73(1-6))

Objective: To determine whether confinement to surfaces treated with juvenile hormone-mimicking compounds would prevent development of the cigarette beetle, (Lasioderma serricorne) or red flour beetle, (Tribolium castaneum).

Methods: Whatman No. 1 filter papers were treated with an acetone solution of juvenile hormone-mimicking compound Zoecon 515 at 10, 30, 100 or 300  $\mu\text{g}/\text{cm}^2$ . Five- to 7-day-old mated or unmated cigarette beetle adults were confined to the surfaces for 24 hrs or less and then transferred to vials containing untreated culture medium. The numbers of eggs, larvae, or emerged adults were then determined.

Results: No  $F_1$  generation resulted from mated cigarette beetle females exposed for 24 hrs to paper treated with Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$ . An 8-hr exposure reduced the  $F_1$  generation over 95% and a 4-hr exposure reduced it over 90%. Most of the insects appeared to have died as embryos. When virgin females were exposed 24 hrs to the treated paper, about 5% of the offspring emerged as adults.

Little effect was noted on the reproduction of cigarette beetle females exposed 24 hrs to paper aged 3 months after treatment with Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$ .

Plans: The relationship between exposure of adult cigarette beetle females to hormone-treated surfaces and the failure of the eggs to hatch when laid in untreated medium will be investigated further.



Interactions Between Beta-Ecdysone and Fat Body In vitro

H. Oberlander and S. M. Ferkovich

(Continuation of report 07 73(1-6))

Objective: In our study of interactions between ecdysone and tissue factors in the insect, we have determined that a factor from the larval fat body stimulates cuticle deposition in Lepidopteran imaginal discs which are incubated in vitro with beta-ecdysone. The focus of the project at this time is on the isolation of the fat body factor.

Methods: Mature final-instar larvae of the Indian meal moth, Plodia interpunctella (Hübner), were used as donors of fat body and wing discs. The tissues were cultured in plastic petri dishes in a modified Grace's tissue culture medium.

Results: The fat body factor which synergizes with beta-ecdysone to enhance cuticle deposition in cultured wing discs, is dialyzable, and insensitive to trypsin digestion. The molecular weight is less than 12,000.

Plans: Fat body conditioned medium is being fractionated in an effort to isolate the fat body factor, chemical identification and mode of action studies will follow.



In vitro Bioassay of Insect Hormones

H. Oberlander and C. E. Leach

(Continuation of report 08 73(1-6))

Objective: To develop sensitive and fast bioassay procedures for structure-activity studies of insect hormones and hormone mimics. Compounds which may have ecdysone, anti-ecdysone or juvenile hormone activity will be evaluated by an in vitro bioassay system.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are incubated in vitro in modified Grace's tissue culture medium with the candidate hormonal agent. The ability of the compound to stimulate or inhibit cuticle deposition is noted.

Results: Insect ecdysones and analogues of these have been tested in vitro. The bioassay was conducted in three ways: continuous exposure to hormone without fat body; continuous exposure to hormone with fat body; and a 24-hr pulse treatment without fat body. The relative activity of the compounds is different for each bioassay method and criterion used. The most consistent results are obtained by using all three bioassay methods with either evagination or cuticle deposition as the criterion of hormone action, rather than only one bioassay method in which both evagination and cuticle deposition are measured.

Plans: The effects of juvenile hormone analogues on ecdysone-induced morphogenesis in vitro are being investigated.



Uptake and Metabolism of  $\alpha$ - and  $\beta$ -Ecdysone by  
Cultured Wing Discs of Plodia  
interpunctella

D. L. Silhacek and H. Oberlander

(Continuation of report 10 73(1-6))

Objectives: Previous studies have provided considerable insight into the physiological mechanism of ecdysone action on cultured wing discs. However, for meaningful interpretation of these findings, it was essential to determine the metabolic fate of ecdysone when added to the tissue culture medium. The purpose of this study was to determine whether ecdysone is converted to other active (or inactive) molecules during the tissue incubations and to measure the extent of ecdysone binding to the wing discs.

Methods: Wing discs were dissected from Indian meal moth larvae and cultured by established methods. Procedures for the quantitative extraction, separation, and measurement of ecdysone and its metabolites were investigated using isotopically labelled hormone.

Results: Current methodology for the extraction and chromatographic separation of ecdysone and its metabolites has not provided the quantitative sensitivity required for these experiments. New procedures using a recently acquired high-pressure liquid chromatograph are being developed. A quantitative procedure for extraction of the ecdysones from the tissue culture medium has not yet been achieved.

Plans: A method for the quantitative recovery of ecdysone and its metabolites from tissue culture medium will be developed. We will examine whether crude protein fractions in the growth medium can be omitted for wing disc development and thereby minimize protein interference with ecdysone extraction. Experiments investigating the metabolism and distribution of tritiated ecdysone during wing disc incubation will be conducted.



Hormonal Control of Chitin Synthesis In vitro

H. Oberlander and C. E. Leach

(Continuation of report 11 73(1-6))

Objective: To investigate the mode of action of ecdysone and juvenile hormone on the initiation and inhibition of metamorphosis, we have developed a model system. Ecdysone stimulates and juvenile hormone inhibits cuticle deposition in imaginal discs in vitro. We are focusing on the action of these hormones on the biosynthesis of chitin.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are cultured in vitro in a modified Grace's medium. The incorporation of D-glucosamine-6- $H^3$  into the discs is evaluated with radio-autographic and scintillation counting methods. The effects of inhibitors of chitin synthesis are evaluated.

Results: Both alpha-ecdysone and beta-ecdysone stimulated uptake of chitin precursor by the discs, but only beta-ecdysone caused marked localization of the isotope in the cuticular region. Polyoxin D, an inhibitor of chitin synthetase, and Cytochalasin B, an inhibitor of sugar transport, prevented ecdysone induced cuticle formation in vitro.

Plans: This in vitro system is being used to investigate the mode of action of juvenile hormone. We will try to determine at what level (precursor uptake, synthesis, deposition) juvenile hormone prevents ecdysone-induced cuticle formation in vitro.



Control of Egg Maturation in Dermestid Beetles

K. W. Vick and J. A. Coffelt

Objective: To elucidate the hormonal control of egg maturation in dermestid beetles including the role of the male in stimulating egg production.

Methods: Black carpet beetle females are dissected and their oocytes measured after mating, decapitation, treatment with cecropia juvenile hormone and other conditions. Published procedures are used for glycogen and protein determinations of the developing oocyte.

Results: We have shown that mating as well as topically applied juvenile hormone stimulates egg production in female black carpet beetles. Egg maturation can be divided into three categories: previtellogenesis, premating vitellogenesis, and post-mating vitellogenesis.

Plans: Studies are being conducted to elucidate the biochemical steps involved in premating and post-mating vitellogenesis.



Culturing Lepidoptera: The Carpenterworm,  
Prionoxystus robiniae

N. C. Leppla, R. E. Doolittle, and J. D. Solomon<sup>1/</sup>

(Continuation of report 14 73(1-6))

Objectives: To develop more efficient rearing methodology and provide research material for cooperating scientists.

Methods: Fertile carpenterworm, Prionoxystus robiniae (Peck) moths, were collected near Stoneville, Ms. and held for oviposition at  $25\pm 2^{\circ}\text{C}$  and  $60\pm 15\%$  RH with a light:dark 8:16 photoperiod. Several hundred of the resulting eggs were shipped to Gainesville, Fla., and placed in the dark at  $27\pm 2^{\circ}\text{C}$  and  $95\pm 5\%$  RH. Larvae, which began to emerge within 10 days, were transferred immediately to containers of diet. These cups contained 10-15 larvae each, and were maintained at  $27\pm 2^{\circ}\text{C}$  and  $50\pm 5\%$  RH with an L:D 14:10 photoperiod.

Larval diets were formulated by incorporating 21 different combinations of the ingredients in 12 types of rearing containers. Each of the substrate materials or nutrients was omitted from at least one batch of medium, and 50-100 larvae were offered each substrate. Since micro-organism inhibitors were inadequate and excess moisture enhanced the growth of toxic contaminants, the "cakes" of diet were removed from the cups and baked at  $60^{\circ}\text{C}$  for at least 5 hrs. The degree of dryness depended on structural characteristics and texture, relative to the ages of larvae. These 4-cm x 7-cm diam "cakes" were placed in fresh rearing containers and infested with the 136 larvae that survived initial screening.

After the entire 90-day development period, cups containing a total of 105 larvae were transferred to a dark environment at  $5\pm 2^{\circ}\text{C}$  and  $85\pm 5\%$  RH to terminate the larval stage. Each week, after the first 30 days, 15 cups of larvae were returned to the  $27\pm 2^{\circ}\text{C}$  incubator and held for pupation. Simultaneously, a second colony was reared according to the optimum conditions established with the first, except 16-oz cups were substituted to improve visibility and a fiberglass coating was added to contain the larvae.

Results: The larval medium now includes pinto beans and alphacel in place of Nuttall oak sawdust. In the 2nd population of carpenterworms reared on this artificial medium, 57% of the larvae matured, 13% pupated, and 13% emerged. Development from egg to adult required an average of 212 days (Table 1). The methods are suitable for large-scale culturing of the carpenterworm at a cost of \$300.00/1000 pupae (assuming 50% yields) and adaptable for use with other wood boring species.



Plans: These new methods are being tested under standardized rearing conditions at Stoneville, Ms.

1/ Forest Service Insect Research Laboratory, P.O. Box 227,  
Stoneville, Ms. 38776

Table 1--Rearing regime for the carpenterworm.

Stage	Environment	Container	Duration (days)
Egg	80 $\pm$ 5°F, 90 $\pm$ 5% RH, 0:24	petri dish	11-13
Larva	80 $\pm$ 5°F, 50 $\pm$ 5% RH, 14:10	transparent cup <sup>1/</sup>	75-82
Post-feeding	40 $\pm$ 5°F, 90 $\pm$ 5% RH, 0:24	transparent cup	65
Post-cold	80 $\pm$ 5°F, 50 $\pm$ 5% RH, 14:10	transparent cup	32
Pupa	80 $\pm$ 5°F, 50 $\pm$ 5% RH, 14:10	transparent cup	19-22
Adult	80 $\pm$ 5°F, 60 $\pm$ 5% RH, 14:10	1 gal. cage	5-15

1/ Plastic 16 oz cup (MC 160) with vented lid (LMC 61) from  
Sweetheart Plastics, Inc.



Culturing Plecia nearctica, the  
"Lovebug"

N. C. Leppla, J. J. Whitesell<sup>1/</sup>, L. C. Kuitert<sup>1/</sup>, and  
J. L. Gillmore <sup>1/</sup>

(Continuation of report 15 73(1-6))

Objectives: To rear lovebugs, Plecia nearctica Hardy, by duplicating natural conditions in the laboratory and conducting basic biological studies. The project is divided into 3 phases: (1) development of adult cages that allow mating, provide suitable oviposition substrates, and promote larval development, (2) determination of the optimum environmental requirements for rearing, (3) adaptation of techniques available for other species that feed on decaying vegetation and (4) simplification of procedures for testing with several generations of lovebugs.

Methods: Flies were field-collected during May 1973 near Gainesville, and 50 pairs were placed in each of 4 isocages (5.4 liter polyethylene boxes with porous paper lids) containing a 2:1 mixture of composted manure and autoclaved sand, covered with dried grass. A mixture of 5% sucrose solution, 2% honey, and 1% methyl para-hydroxybenzoate was provided in 15 cc glass vials stoppered with cotton inserts, and the cages were incubated at  $28 \pm 1^\circ\text{C}$  and  $80 \pm 5\%$  RH with a light:dark 14:10 photoperiod. Water was added to bind components and form a moist substrate. Mating occurred continuously, and eggs deposited within the 5-7 day adult period were surface-sterilized, formed into 19 masses, and individually transferred to 16 oz plastic cups provisioned with the organic substrate. Larval and pupal development were recorded and the emergent flies were transferred to fresh containers.

Results: In the 19 isolated groups of larvae, 6 developed normally, 4 were arrested in the 5th instar, and 9 were destroyed by microorganism contamination. Flies from the larval colony were accumulated in 4 containers, and 2 of these produced viable eggs. Field-collected pairs had a mating frequency of 1.76 spermatophores/mated female, the first generation of laboratory-reared adults transferred an average of 1.55, and optimum conditions resulted in 2.0. Similarly,  $F_1$  flies produced 293.44 eggs/mated female,  $F_2$  adults averaged 190.18, and in nature 300-350 is common. Larval development required 63-100 days in the laboratory, and the mean generation time was 126.5 for the  $F_1$  and 122.4 for the  $F_2$  populations.

Plans: The study should be continued through several generations to refine the techniques and provide practical procedures for maintaining this species in the laboratory.

<sup>1/</sup> A project of the Department of Entomology and Nematology, University of Florida, N. Leppla cooperating.



Improved Laboratory Culture of Opius longicaudatus (Ashm.)

P. D. Greany, T. R. Ashley, and D. L. Chambers

Objectives: To optimize biotic and abiotic conditions affecting laboratory rearing of Opius longicaudatus, a parasite of tephritid fruit fly larvae.

Methods: Three artificial diet formulations were evaluated relative to their acceptability to larvae of the Caribbean fruit fly, and to the ease of acquisition of hosts of the optimal developmental stage for parasitization. Fully-grown and younger host larvae were compared for oviposition and development.

Results: Substantial improvements were made in the rearing of Opius longicaudatus. A method was developed to conveniently acquire larvae of the optimal stage for oviposition and development, and an efficient adult collection technique was developed. Fully-grown larvae, while equally acceptable for oviposition, produce ca. 4 times fewer parasites than equal numbers of larvae 1-2 days younger. A semisolid (agar) diet and a bagasse (sugar cane fiber) diet both were rejected in favor of a corn cob grit-based diet, which more easily allowed differential separation of the diet from immature larvae by flotation. The larvae are concentrated, provided with fresh diet, and are confined between cloths in an embroidery hoop for a 24-hr exposure period. Nearly 100% parasitization ordinarily results, and the production of mostly parasites rather than flies greatly simplifies eventual collection.

For collection of adult parasites, a bottomless sleeve cage is placed over a tray of puparia just before the date of emergence. Upon emergence, the parasites climb to the upper portion of the unit, a sliding partition is inserted, CO<sub>2</sub> is injected, and moments later the parasites can be collected very quickly. Fewer than 5% of the emerged parasites escape initial collection. None of the puparia are subjected to CO<sub>2</sub> in this process.

Plans: Intended studies include definition of optimal temperature, humidity, light quality and intensity, and photoperiod regimes for each stage of the parasite. Studies on the optimal spatial density and air exchange rates and on the host-parasite ratio are planned, as are adult nutritional studies. The effects of adult transfer procedures (CO<sub>2</sub>, N<sub>2</sub>, aspiration, chilling) are to be evaluated. Criteria are to be developed for assessment of the quality of mass-produced, laboratory-reared parasites.



Parasite-Vectored Transmission of Bacterial  
Pathogens of the Caribbean Fruit Fly

P. D. Greany, G. E. Allen, D. L. Chambers, and L. Greany

Objectives: To describe the role of Opius longicaudatus (Ashm.) as a vector of pathogens of the Caribbean fruit fly, Anastrepha suspensa (Loew), and to discover means of reducing the incidence of infection in laboratory cultures.

Methods: Comparisons of larval and puparial survival of parasitized and nonparasitized hosts were made. Parasitized moribund and dead Caribbean fruit fly larvae and puparia, and adult parasites, were examined microscopically for anatomical symptoms of disease. Dead insects were surface sterilized and then triturated in sterile saline. The suspensions were streaked on nutrient agar plates incubated aerobically.

Bacteria found growing on the plates were identified by Dr. G. E. Allen of the Department of Entomology, University of Florida, and by L. Greany of the Veterans' Administration Hospital in Gainesville, Florida, by a series of morphological and biochemical characters. Sensitivities of the bacteria to an array of antibiotics were also determined.

Results: Significant differences in larval and puparial survival between parasitized and nonparasitized hosts were observed. Three species of potentially pathogenic bacteria were isolated, including Serratia marcescens, Pseudomonas aeruginosa, and Proteus morgani. In addition, Staphylococcus epidermidis and a Micrococcus sp. were isolated. Insects infected by S. marcescens were easily recognized as they acquired a bright pink coloration.

Pseudomonas aeruginosa proved to be resistant to all antibiotics tested other than Polymyxin B sulfate and Colistin. Serratia marcescens and P. morgani were sensitive to Chloromycetin (Chloramphenicol), as were S. epidermidis and the Micrococcus sp.

Plans: Efforts will be made to fulfill Koch's postulates by intrahemocoelic injection of pure cultures of each bacterial species, and by reisolation of the specific bacteria from dead insects. Ovipositors from parasite females will be excised and cultured on nutrient agar plates to determine the potential for host infection during oviposition. Polymyxin B sulfate will be tested for use in decontaminating fly eggs destined for parasite production, and Chloromycetin will be incorporated into the larval diet to inhibit growth of pathogenic bacteria. Efforts will be made to note any deleterious effects resulting from use of the antibiotics, such as adverse effects on symbiotic microorganisms which may normally accompany host larvae.



Effect of Host Developmental Stage on Oviposition  
and Development of Opius longicaudatus (Ashm.)

P. O. Lawrence<sup>1/</sup>, P. D. Greany, and R. M. Baranowski<sup>2/</sup>

Objectives: To define the ovipositional and developmental suitability of larvae of the Caribbean fruit fly, Anastrepha suspensa (Loew), for attack by Opius longicaudatus, and to investigate factors leading to physiological unsuitability when encountered.

Methods: Larvae of specific developmental stages were exposed to attack by O. longicaudatus females. Samples of exposed larvae were dissected to determine the incidence of oviposition. Other larvae were allowed to develop for determination of effective parasitism rates.

Results: No significant difference in oviposition rates was observed between fully-grown larvae and nearly-mature larvae, although very young larvae often escaped parasitization. However, a highly significant difference was noted in rates of effective parasitism for fully-grown and nearly mature larvae, with ca. 4 times more parasites being produced from the younger larvae.

Plans: Intended studies include determination of the relationship of inter- and intrastadial developmental status to effective parasitism. Ligation experiments will be conducted to determine the relationship of endocrinological events to host suitability.

<sup>1/</sup>,<sup>2/</sup> Graduate Student and Professor, respectively, Department of Entomology and Nematology, University of Florida, Gainesville, Fla.



Culturing Lepidoptera: Calcium Alginate  
vs HWG in Cabbage Looper Diet

N. R. Spencer<sup>1/</sup> and N. C. Leppla

(Continuation of report 17 73(1-6))

Objective: To successfully adapt alginates as gelling agents in artificial diets for Lepidoptera.

Methods: Calcium alginate does not require cooking and allows the medium to remain viscous for comparatively long periods; therefore, it would be advantageous to substitute the material for agar or carageenan (HWG) in cabbage looper diet. A cooked diet containing HWG and an uncooked medium with calcium alginate substituted for HWG were formulated and balanced. The pinto bean diet was modified by adding water and  $\text{CaSO}_4$ , buffered with  $\text{Na}_4\text{P}_2\text{O}_7$ , and blended at a high speed. Thus, essentially identical nutrients with different physical characteristics were tested with 4 generations of cabbage looper larvae.

Results: A "cold" diet containing the substituted alginate has been formulated and is currently being tested. There were no statistically significant differences in the percentage hatch, survival, larval development, pupal weight or adult longevity and fecundity between insects reared on the two substrates. Calcium alginate costs approximately 1/3 less than HWG, however 1/3 more is used in the diet.

Plans: The study will be conducted until the calcium alginate medium has been tested under standardized rearing conditions with a large cabbage looper colony.

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Gainesville, Fla.



Effects of Nitrogen and Gamma Irradiation of Pupae  
of Anastrepha suspensa on Emergence, Longevity,  
Fecundity, and Sterility

J. L. Sharp, T. R. Ashley, D. R. Bennett, and B. J. Smittle<sup>1/</sup>

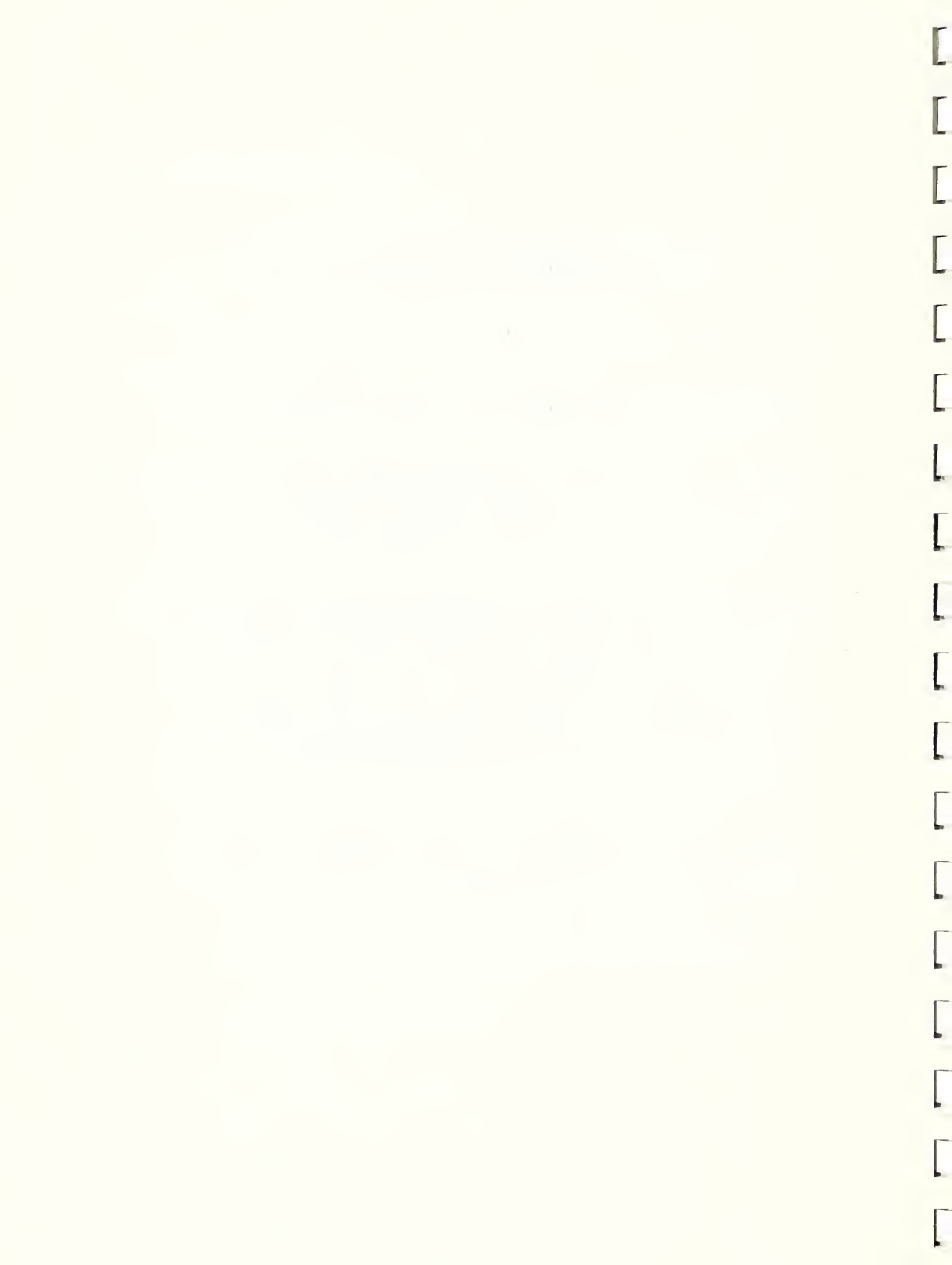
Objectives: To determine the effects of irradiation in nitrogen of A. suspensa pupae on emergence, longevity, fecundity, and sterility of adults.

Methods: Laboratory-reared A. suspensa pupae from Homestead, Fla. were irradiated with <sup>60</sup>Co 2 days before adult eclosion in nitrogen atmosphere with 2, 3, 4, and 5 kiloröntgen (kR) at a dose rate of ca. 1 kR/min. Controls were not irradiated but were exposed to nitrogen.

Results: Nitrogen exposure alone or nitrogen plus irradiation had no significant effects on emergence of adults, whether pupae were exposed to nitrogen for 3.5 hr or exposed to nitrogen for 0.5 hr and irradiated with 10 kR. Doses from 2 to 5 kR tend to increase longevity, reduce fecundity, and reduce or prevent oviposition and hatching of eggs. Males from pupae exposed to nitrogen and irradiated with 5 kR and mated with normal females yielded 99.9% sterility; females from pupae exposed to nitrogen and irradiated with 5 kR and paired with normal males did not oviposit.

Plans: This aspect of the research has been completed; however, further studies are contemplated using emergence, longevity, fecundity, and sterility measurements to indicate the quality of laboratory-reared or treated insects.

<sup>1/</sup> Research Entomologist, Insects Affecting Man Research Laboratory, Gainesville, Fla.



18 73(7-12)

Micro Techniques and Analytical Methods

R. R. Heath and J. H. Tumlinson

(Continuation of report 18 73(1-6))

Objective: To develop analytical techniques enabling elucidation of the structure of compounds in microgram quantities, thus, requiring fewer insects.

Methods: During the past 6 months, emphasis has been placed on obtaining carbon-13 NMR spectra on microgram quantities. Most of the techniques are similar to those reported in the previous semi-annual report (18 73(1-6)).

Results: We have been able to obtain good  $^{13}\text{C}$  NMR spectra with about 800  $\mu\text{g}$  of standard compounds.

Plans: More work is needed to reduce the amount of material necessary to obtain  $^{13}\text{C}$  spectra. We also plan to learn techniques necessary to obtain partially relaxed fourier transform  $^{13}\text{C}$  NMR spectra on microgram quantities.



Pheromones of the Lesser Peachtree Borer Synanthedon pictipes  
(Grote & Robinson) and the Peachtree Borer Sanninoidea exitiosa (Say)

J. H. Tumlinson, C. E. Yonce<sup>1/</sup>, R. E. Doolittle,  
R. R. Heath, C. R. Gentry<sup>1/</sup>, and E. R. Mitchell

(Expansion and continuation of report 21 73(1-6))

Objectives: As part of a program to develop an integrated pest management system for peach insects, the sex pheromones produced by the female lesser peachtree borer and peachtree borer will be isolated, identified, synthesized, field tested, and incorporated into survey and control programs for these insects.

Methods: For isolation, identification, and bioassay methods see 21 73(1-6). The four geometrical isomers of 3,13-octadecadien-1-ol acetate were synthesized. Final purification of the synthesized pheromones was accomplished by high pressure liquid chromatography on silver nitrate treated silica gel using benzene as a solvent.

Results: (E,Z)- and (Z,Z)-3,13-octadecadien-1-ol acetate were identified as the sex pheromones of the lesser peachtree borer and peachtree borer, respectively. The synthesized compounds, when highly pure, elicited the same response as the natural compounds in the field, and are highly attractive to males of the respective species in field tests. Additionally, a mechanism for species isolation was discovered that may eventually be useful for control by disruption of the insects' communications system. Briefly, the pheromone of each species inhibits the response of males of the opposite species to their own pheromone.

Plans: The two pheromones are being synthesized and purified in quantities large enough to support extensive field tests during the 1974 season. These tests, to be conducted in Florida, Georgia, and Wisconsin (by Dr. Karandinos), will include release-recapture studies, dispersal, trap design and placement, and other similar studies. Additionally, all the isomers of these compounds are being provided to Dr. David Nielsen, Wooster, Ohio for testing on other Sesiidae species.

Publications: See App. 2 Journal Review: Yonce, C. E., J. H. Tumlinson, C. R. Gentry, and E. R. Mitchell; and Tumlinson, J. H., C. E. Yonce, R. E. Doolittle, R. R. Heath, C. R. Gentry, and E. R. Mitchell.

<sup>1/</sup> Research Entomologists, Southeastern Fruit & Tree Nut Research Station Laboratory, P.O. Box 87, Byron, Ga.

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Isolation and Identification of the Sex Pheromone of the  
Khapra Beetle, Trogoderma granarium

K. W. Vick

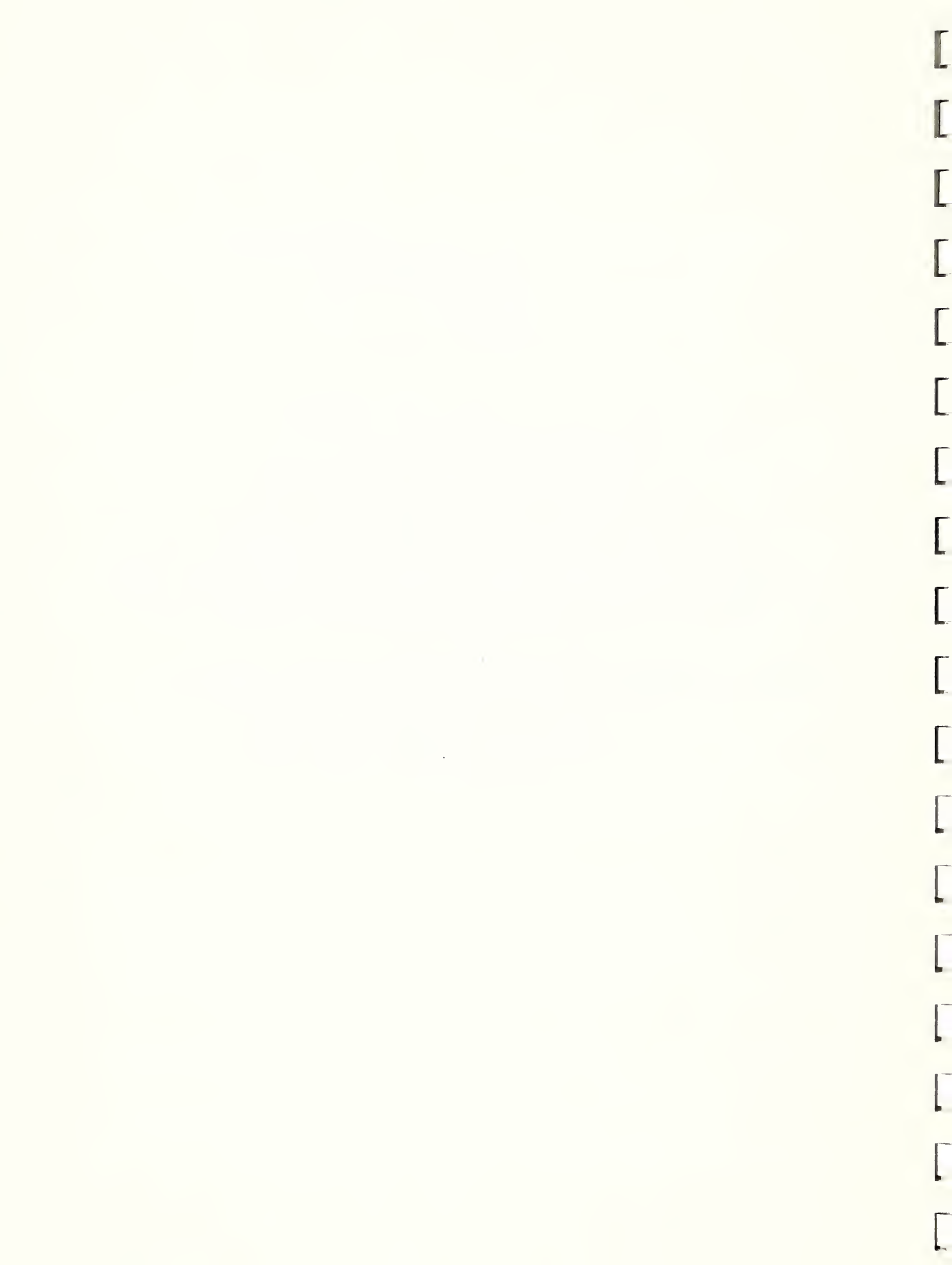
(Continuation of report 22 73(1-6))

Objective: To isolate and identify the sex pheromone of the khapra beetle using a novel bioassay technique.

Methods: Since the khapra beetle is not allowed in this country, a method of bioassay not based on khapra beetle males had to be developed. Since T. inclusum had previously been shown to be responsive to khapra beetle female sex pheromone extract, the decision was made to use T. inclusum males as the bioassay animals with the standard T. inclusum bioassay method. Female khapra beetles were collected over a two-year period in India and extracted with diethyl ether. This extract was then sent to this laboratory for purification and identification.

Results: Several fractions with sex pheromone activity have been partially purified by liquid and gas chromatography. These fractions have been sent to India for bioassay against khapra beetle females.

Plans: Work will continue toward chemical identification of the khapra beetle female sex pheromone.



A Potent Sex Attractant for the Carpenterworm Moth,  
Prionoxystus robiniae

R. E. Doolittle, J. D. Solomon<sup>1/</sup>, W. L. Roelofs<sup>2/</sup>, M. Beroza<sup>3/</sup>,  
M. McKnight<sup>4/</sup> and A. Tagestad<sup>5/</sup>

(Continuation of report 23 73(1-6))

Objectives: Isolate, identify and synthesize the female produced sex pheromone of the carpenterworm moth and evaluate its usefulness as a survey and/or control tool for the insect.

Methods: Abdominal tips of the female insects and filter papers on which female insects had crawled were extracted, and these extracts purified chromatographically. The electroantennogram response of males to a series of mono-unsaturated fourteen carbon acetates was used to determine the possible position(s) of unsaturation in the pheromone. The acetates of four isomeric diolefinic fourteen carbon alcohols were synthesized and field tested for their attractancy to males.

Results: During the 1973 emergence season, a new trap was designed that proved to be much more efficient than the old design. (Z,E)-3,5-tetradecadien-1-ol acetate was very attractive and up to 100 males were caught in one trap over a five-hour period. Admixture of up to 50% of (E,E)-3,5-tetradecadien-1-ol acetate and other mono-unsaturated acetates did not significantly reduce the attractiveness of the (Z,E) isomer. Prionoxystus males were caught in field tests run in shelterbelt forests in North Dakota. In addition, another carpenterworm, Acosus centerensis (Litner), the aspen carpenterworm, was caught by the synthetic lure.

Plans: A final batch of the synthetic (Z,E) compound is being prepared for the 1974 emergence season. More efficient synthetic routes to the (Z,E) isomer will be investigated in order to make production more attractive commercially to several chemical companies. During the 1974 emergence season, a small field control experiment will be conducted in Stoneville. Efforts will be continued to establish the identity of the natural pheromone as (Z,E)-3,5-tetradecadien-1-ol acetate.

<sup>1/</sup> Forest Service Insect Research Laboratory, P.O. Box 227, Stoneville, Ms. 38776. <sup>2/</sup> New York Agricultural Experiment Station, Geneva, N. Y. 14456. <sup>3/</sup> Organic Chemical Synthesis Laboratory, Agricultural Environmental Quality Institute, Beltsville, Md. <sup>4/</sup> Division of Forest Insect & Disease Research, Forest Service, Washington, D. C. <sup>5/</sup> Shelterbelt Laboratory, Bottineau, N. D. 58318.



## Sex Pheromone Studies of the Navel Orangeworm

J. A. Coffelt, K. W. Vick, and L. L. Sower

(Continuation of report 26 73(1-6))

Objectives: To isolate and identify the female sex pheromone of the navel orangeworm, and ultimately, to evaluate its potential use as a control or detection tool for wild populations infesting either wild or cultivated host materials. Therefore, we must develop an understanding of the physiological, behavioral and environmental factors which may influence the utility of the pheromone as a tool in the integrated control of this species.

Methods: Laboratory bioassays based upon male activation response were used to monitor chemical isolation of the pheromone. The influence of male age and/or prior use in bioassay upon subsequent responsiveness were determined by exposing males of different ages and of different bioassay histories to standard quantities of crude pheromone extract. Crude pheromone was obtained by collecting filter papers upon which virgin females were held for 24 hrs. Filter papers were changed daily for 4 days. Crude material was extracted from the filter paper by use of a soxhlet apparatus. Initial purification was achieved principally by means of gradient elution liquid chromatography (LC). Three successive LC runs, using different column materials, were employed. Active areas from each run were determined by bioassay. Subsequent to the LC runs, the active fractions were combined and further purified by means of preparative gas chromatography. Successful recovery of pheromone activity from gas chromatography required the use of an all-glass GC system.

Results: Two, 3, and 4-day-old males were similar in pheromone-responsiveness, and all were significantly more responsive than were 1-day-old males. Male response to pheromone declined after 4 days of age. No significant reduction in male responsiveness was noted when males were used more than 1 time for bioassay provided that at least 24 hrs elapsed between tests. Peak male responsiveness to the pheromone was recorded during the last 1/3 of the 12-hr scotophase. A single peak of pheromone activity was obtained in each of the 3 LC runs, and preliminary assays of the fractions collected from GC suggest a single peak of activity.

Plans: We will continue to process female moths for pheromone collection, conduct initial purification steps, and continue the investigation of physiological, environmental and behavioral parameters which influence male response to, and female production of the pheromone.



Isolation and Identification of Attractants Mediating  
Host-Finding by Opius longicaudatus (Ashm.)

P. D. Greany, J. H. Tumlinson, D. L. Chambers,  
D. R. Sukkestad, and G. M. Boush

(Continuation of report 66 73(1-6))

Objective: To define the mechanisms used in host-finding by Opius longicaudatus, a parasite of tephritid fruit fly larvae.

Methods: Extracts were prepared from fresh peaches, non-infested rotting peaches, and from peaches infested with the Caribbean fruit fly. Bioassays were conducted in the greenhouse. Distillation of active extracts was followed by gas chromatography using a Porapak-Q column. Studies on the role of microorganisms in attraction were conducted in cooperation with Dr. G. M. Boush of the University of Wisconsin. Extracts of pure fungal cultures on fruit and on synthetic media were prepared and bioassayed.

Results: Extracts of infested and non-infested but rotting fruit were equally attractive, while extracts of fresh fruit were unattractive. Only O. longicaudatus females were attracted. Extracts using polar solvents were more active than those with non-polar solvents. Gas chromatography of active distillate revealed the presence of only one compound, ethanol. Bioassay of pure ethanol yielded attraction, but the level of response was less than expected when tested at rates equivalent to the ethanol concentration of crude active extracts indicating the possible involvement of other agents. Acetaldehyde was also bioassayed and was found to be appreciably more active than ethanol. Structure-activity bioassays were conducted with all 1-, 2-, and 3-carbon alcohols, aldehydes, and acids. No significant response was noted to compounds other than ethanol and acetaldehyde, even at high concentrations.

The fungus Monolinia fructicola, which causes brown rot of peaches, was isolated from rotting peaches. Cultures of this organism on fruit and on synthetic media yielded attractive crude extracts, as did Penicillium digitatum. These studies indicated that host-finding by O. longicaudatus is mediated at least in part by a response to the odor of rotting fruit, irrespective of the presence of host larvae.

Plans: Field testing of ethanol and acetaldehyde alone and in combination is anticipated, and will be conducted in the Homestead, Florida, area. Studies on the influence of ovarian maturation upon responsiveness to attractants are planned. Further studies on perception of host larvae within infested fruit are being conducted.



# White Peach Scale Behavior

J. R. McLaughlin, T. R. Ashley, and D. L. Chambers

Objective: To describe pre-mating and mating behavior of the white peach scale, Pseudaulacaspis pontagona, with emphasis on the role of pheromones.

Methods: Newly-emerged male scales were introduced into the downwind end of a Y-tube olfactometer (6-in. diam. plexiglass, 24-in. stem and 36-in. arms). Room air (80°F, 50% RH, 0.25 m/sec in stem) was drawn across a potato (control) at the mouth of 1 arm of the tunnel and a potato supporting a colony of mature, virgin female scales at the other arm. After 15 min, the position of the potatoes was reversed and at subsequent 5 to 15 min intervals observations were made with potato vs. room air, room air alone, and again with potato vs. potato plus scale.

Results: Males flew from the congregation point on the screen at the downwind end of the olfactometer to the female infested potato. This flight usually was very direct. The males exhibited little upwind movement in air streams with no treatment or with potato alone. Reintroduction of a female-infested potato initiated further upwind flight by males.

Plans: Upwind flight by males will be the laboratory test criterion for screening the attractancy of volatiles obtained from female scales and potatoes and candidate synthetic compounds. The biological significance of the observed attraction will be investigated and quantified.



Use of Sex Pheromones for Behavioral Control  
of Loopers and Related Noctuid Species

J. R. McLaughlin, E. R. Mitchell and D. L. Chambers

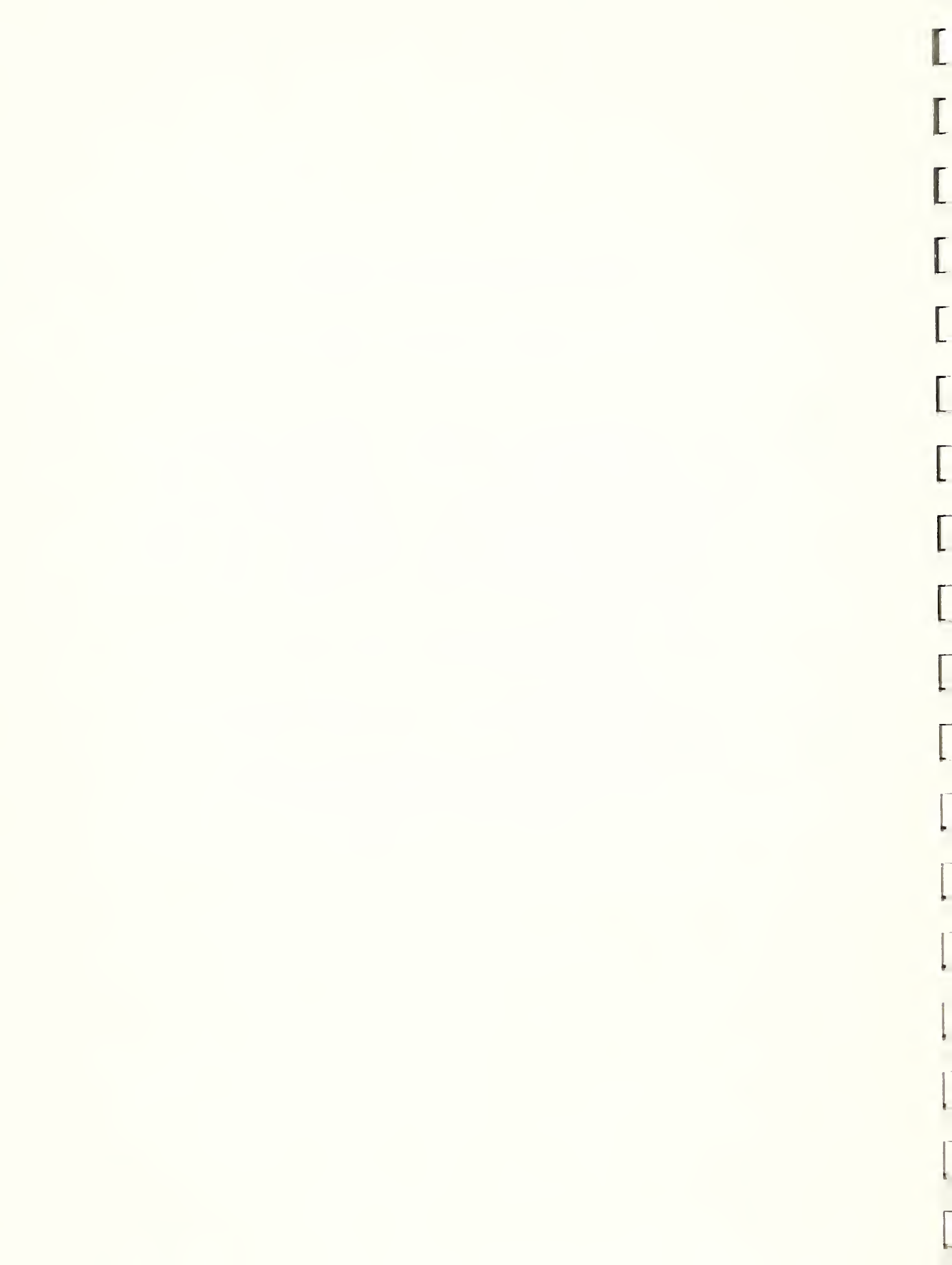
(Continuation of report 33 73 (1-6))

Objectives: This study explores methods for controlling population levels by manipulating the sex pheromone communication systems of the cabbage looper, soybean looper, and several related species. The technique whereby the sex pheromone is continually evaporated into the air over a test area at a concentration above the male behavioral threshold (Environmental Permeation) is being examined. To adequately test this technique against wide-ranging species such as the cabbage and soybean loopers, large land areas must be treated. We are developing a system for broadcasting the pheromone over such areas.

Methods: We have field-tested string, wood chip, oil, and clay-based polyeric gels as carriers for the pheromone.

Results: The cellulose-matrix carriers impregnated with pheromone and broadcast in small field plots were effective in disrupting mating communication in cabbage loopers and soybean loopers. They could be utilized in a 14-day-interval broadcast program to permeate an area with pheromone. The rate of pheromone expenditure would be ca. 2.25 ml/ha per treatment. Oil and gel spray formulations were ineffective.

Plans: Field testing of microencapsulated spray formulations has just begun.



Effect of the E Isomer on the Attractancy of  
Z-7-Dodecenyl Acetate

J. R. McLaughlin, E. R. Mitchell and M. Beroza<sup>1/</sup>

Objective: To evaluate the effect of the E isomer on the attractiveness of the noctuid sex pheromone Z-7-dodecenyl acetate.

Method: Can-type traps were baited with 1.25 ml polyethylene vials containing 25  $\mu$ l of a given mixture of Z- and E-7-dodecenyl acetates (dda). The 1st test, conducted in September 1973 in soybean fields, consisted of 3 randomized complete blocks of baited traps (60 m between traps and not less than 400 m between blocks). The 2nd test was conducted in February 1974 in cabbage fields and consisted of 2 randomized blocks with rotation of bait plus trap from one trapping position to the next after each trapping interval.

Results: The E isomer did not act as a synergist or inhibitor of attraction for males of the soybean looper, Pseudoplusia includens, or cabbage looper, Trichoplusia ni. The E isomer did not attract any species of moth in north Florida. Other noctuid species attracted are routinely attracted by Z-7-dodecenyl acetate.

Plans: Future specifications for the looper pheromone may be less exacting in regard to E isomer content. The E isomer may be used as a diluent to control the rate of release of the pheromone in certain experiments.

<sup>1/</sup> Organic Chemical Synthesis Laboratory, Agricultural Environmental Quality Institute, Beltsville, Md.



Control of Stored-Product Lepidoptera  
With Sex Pheromone

L. L. Sower, K. W. Vick and J. A. Coffelt

(Continuation of report 32 73(1-6))

Objectives: To make sound recommendations as to whether or not certain lepidopterous pests of stored products can be directly controlled by introducing synthetic sex pheromone into their environment. The immediate objectives are to determine the effects of pheromone concentrations and population densities on the mating frequencies and behavior of Indian meal moths.

Methods: Tests were conducted in small containers ranging from 500 ml to 8 cu ft in 20- x 20- x 8-ft rooms. Mating frequencies and behavior were monitored at several pheromone concentrations and population densities. Pheromone release patterns of virgin females were determined by observation and the pheromone release rates at different ages and times of day were measured.

Results: A dose/response curve for the pheromone concentration/mating frequency relationship at a moderate population density of 125 pairs of insects per 20- x 20- x 8-ft room is shown in Fig. 1. At higher densities, 30 pairs per 2- x 2- x 2-ft container, similar pheromone concentrations had much less effect. At constant pheromone levels mating frequencies appeared to be inversely proportional to the population density.

At high population densities (>1 pair per sq. ft. of wall surface) normal males virtually always located calling females and copulated within 1 min. Males habituated to synthetic pheromone completely ignored calling females and seldom copulated within 30 min; however, around 30-40% of these insects copulated within 24 hrs.

Observational data suggest that male insects occasionally become sexually active without apparent stimulation by pheromone. Such males appear to locate females by random movement alone where high population densities occur. Unmated females call more than 50% of the time releasing ca. 0.1 ng of pheromone per min.

Several aldehydes at high concentrations were found to stimulate response from males that are quite similar to the sex pheromone responses. At dosages of ca. 1 mg/l of air some aldehydes were toxic.

Plans: The population density/pheromone concentration studies will be continued until a reliable relationship is established. Similar studies will be initiated with the Angoumois grain moth when sufficient synthetic pheromone is obtained.



Fall Armyworm: Inhibition of Pheromone Perception  
by Males With Synthetic Acetates

E. R. Mitchell, W. W. Copeland, C. A. Morgan  
A. N. Sparks<sup>1/</sup>, A. A. Sekul<sup>1/</sup>

(Continuation of report 38 73(1-6))

Objective: To evaluate the effects of synthetic acetates on sex pheromone communication in the fall armyworm (FAW).

Methods: The disruptive effect of Z-7-dodecen-1-ol acetate (Z-7-dda), Z-9-dodecen-1-ol acetate (Z-9-dda), and (Z,E)-9,12-tetradecadien-1-ol, acetate ((Z,E)-9,12-tda) on pheromone communication in the FAW was assessed by determining whether males could locate pheromone-releasing females in small field plots when they were simultaneously exposed to pheromone-impregnated atmosphere. Two experimental areas (ca. 150 m apart) were established in a soybean field adjacent to plots of field corn. Z-7-dda, Z-9-dda, or (Z,E)-9,12-tda was evaporated into the air of 1 plot from 16 dispensers (1.25 ml polyethylene vials each containing 0.1 ml chemical) arrayed at 3-m intervals in a 4 x 4 checkerboard pattern. The dispensers were positioned on wooden stakes at or slightly above the top of the vegetation (ca. 1 m). The position of the treated and untreated plots was reversed at 1-2 day intervals until there were 3 or 4 complete rotations through each location. A cylindrical electric grid trap baited with 6 virgin female FAW (2 day old) was positioned in the center of each plot. Bait females were replaced every 3 or 4 days. Captured insects were collected and counted daily.

Results: Atmospheric permeation with Z-9-dda and (Z,E)-9,12-tda effected a greater than 85% disruption of communication between male and female FAW (Table 1). The results suggest that these chemicals might be used to successfully interrupt the normal reproductive cycle of the FAW in an area-wide suppression program.

The trap in the plot treated with Z-7-dda captured more than twice the number of moths captured in the corresponding control trap (Table 1). This may be an artifact; however, moth captures were consistently greater (7 out of 8 trapping periods) in the treated plot. The reasons for this apparent synergistic effect are under investigation but as yet are unknown.

Plans: Continue to study the effects of various synthetic chemicals on the behavior of the FAW.

Publications: See App. 2 NTE: E. R. Mitchell, W. W. Copeland, C. A. Morgan, A. N. Sparks, A. A. Sekul.

<sup>1/</sup> Research Entomologist and Research Chemist, respectively, Southern Grain Insects Research Laboratory, Tifton, GA.

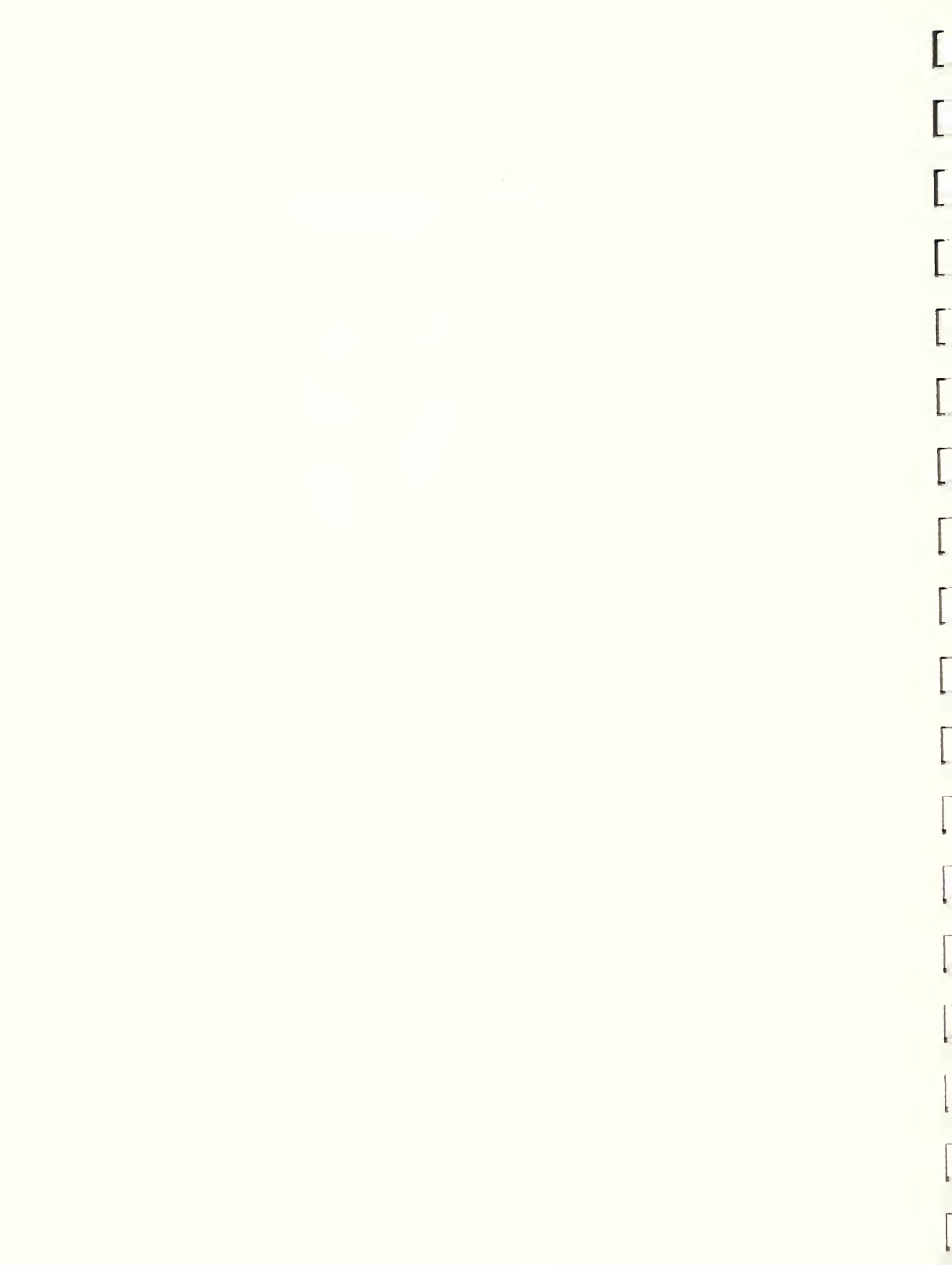


Table 1.--Percent reduction or increase in captures of fall armyworm males in female-baited traps placed within grids of pheromone evaporators. Gainesville, Fla., Aug. 31-Sept. 28, 1973.

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Pheromone	Trap nights	Mean no. ♂ captured/ trap per night		% reduction or increase
		Control plot	Treated plot	
<u>Z</u> -7-dodecen-1-ol acetate	8	12.0	26.5	+120.1
<u>Z</u> -9-dodecen-1-ol acetate	7	463.6	65.4	- 85.9
( <u>Z</u> , <u>E</u> )-9,12-tetradecadien- 1-ol acetate	7	36.7	2.7	- 92.6

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### Pheromone Dispensing Equipment

J. M. Stanley, E. R. Mitchell, J. R. McLaughlin

Objective: Large scale pest management systems that include pheromones as an element will require equipment for its distribution. The applicability of equipment that is available will be investigated.

Methods: Personnel that have conducted research and developed equipment for aerial application will be contacted. Consideration will be given to cooperative use of any equipment that might be available for further research.

Results: Several telephone conversations were made with Mr. L. G. Schoenleber, Agricultural Engineer, Western Region, ARS, Yakima, Washington, concerning equipment that he has developed. A string dispensing unit has been obtained on loan and will be evaluated. Plans for a ULV spray system have also been received from personnel at Yakima. Consideration is being given to modifying this unit for research on the dispensing of encapsulated materials.

Plans: Further searching for available equipment that offers promise will be made and preliminary testing will be conducted.



Morphology and Histology of the Compound Eye  
of the Caribbean Fruit Fly

H. R. Agee and M. L. Park

Objective: To determine the morphology and histology of the compound eye of the Caribbean fruit fly, Anastrepha suspensa (Loew).

Method: The compound eyes of the fruit fly will be examined with the stereomicroscope and fixed and stained sections will be studied using the light microscope to determine the cellular structure of the ommatidium units.

Results: Each compound eye is composed of approximately 10-12,000 ommatidia. Each ommatidia is 20 micrometers wide at the lens and 180 micrometers long from lens to basement membrane. The rhabdom extends from the base of the pseudocone to the basement membrane.

Plans: Additional histological data will be derived to establish the correct location and depth for implanting microelectrodes for determining the spectral sensitivity of the compound eye.



Spectral Sensitivity of the Compound Eye  
of the Caribbean Fruit Fly

H. R. Agee and J. C. Webb

Objectives: To determine the spectral sensitivity of the compound eye of the Caribbean fruit fly, Anastrepha suspensa (Loew) and relate these findings to behavior of the organism.

Methods: A calibrated light stimulus system capable of presenting monochromatic pulses of light energy ( $\text{microwatts/cm}^2$ ) will be used to stimulate the compound eye of the caribfly. Special microelectrodes and electrophysiological techniques will be used to detect and record the spectral sensitivity of the compound eye to light stimuli at wavelengths from 350 nm to 1,500 nm.

Results: The light system has been calibrated and instrumentation developed to determine the spectral sensitivity of the caribfly.

Plans: This project will be completed within 6 months and information obtained will be used to plan and conduct behavioral tests using selected colors to attract the caribfly.



Influence of Color and Intensity of Light on Flight  
and Mating Activity of Cabbage Looper Moths

W. K. Turner and N. C. Leppla

(Continuation of report 38 73(1-6))

Objective: To study the influence of color and nocturnal intensity of light on the flight and mating activity of the cabbage looper, Trichoplusia ni (Hübner).

Methods: Populations of 50 male and 50 female (1-2 day old) cabbage looper moths were held for 2 days under various colors and nocturnal intensities of light. Twelve treatments were applied consisting of four nocturnal intensities for each of three colors, green, red and ultraviolet (BLB), with a single combination of color and nocturnal intensity used in each test. Fluorescent lamps were used and intensities were made equal by neutral density filtering. Nocturnal levels of 0, 1, 10 or 100% of the diurnal intensity were obtained with a cam operated shutter, which provided gradual transitions to stimulate natural conditions. Nocturnal and diurnal temperatures were 76 and 84°F, respectively. CO<sub>2</sub> produced by the moths was monitored during the final 24 hrs of the tests and served to indicate flight activity. Data on mating included percent of females mated, and spermatophores per mated female.

Results: Figure 1 shows the relative flight activity for the various treatments. Darkness following ultraviolet resulted in unusually low nocturnal activity. (The corresponding data of the earlier report was erroneous due to a light leak (ca. 1%) during the nocturnal period). Only the continuous green light inhibited nocturnal activity. Mating was inhibited by green light but not by red or ultraviolet.

Plans: This work has been terminated: a manuscript is in progress.



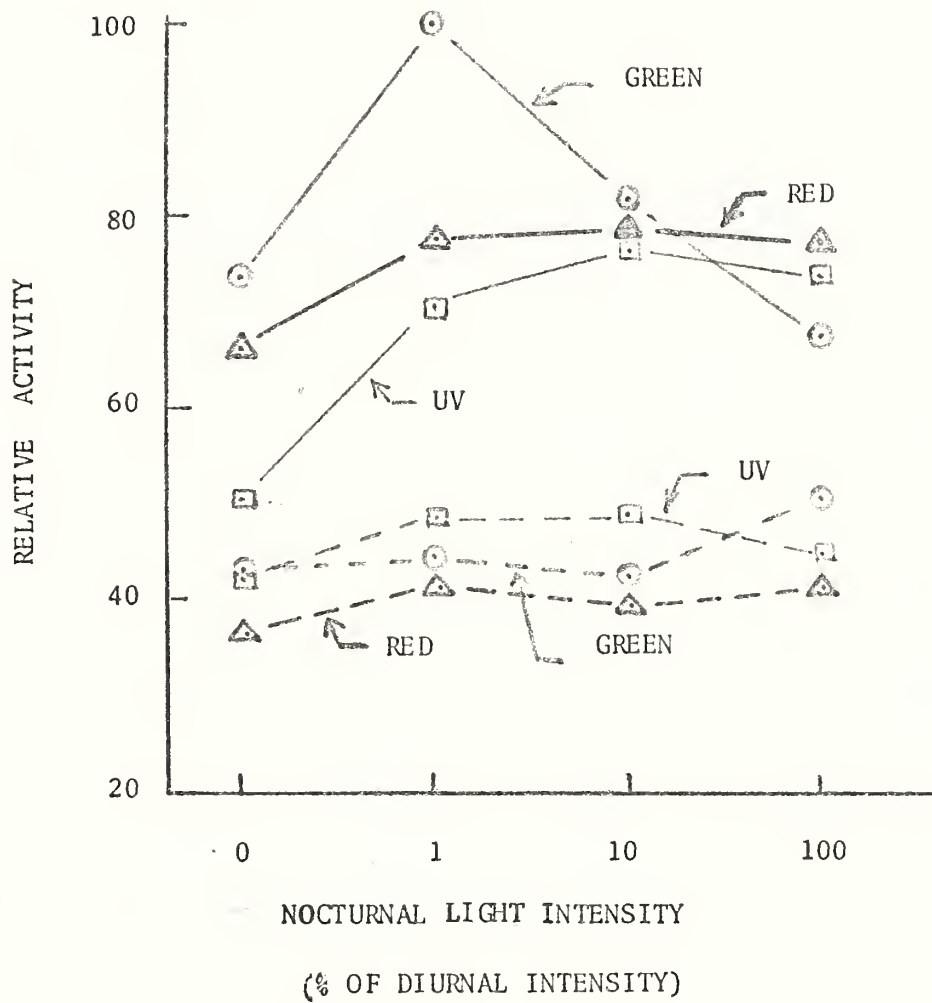


FIG. 1. ACTIVITY OF CABBAGE LOOPER MOTHS UNDER 3 COLORS OF LIGHT AND 4 INTENSITIES OF NOCTURNAL LIGHT. NOCTURNAL ACTIVITY INDICATED BY SOLID LINES, DIURNAL ACTIVITY BY BROKEN LINES.



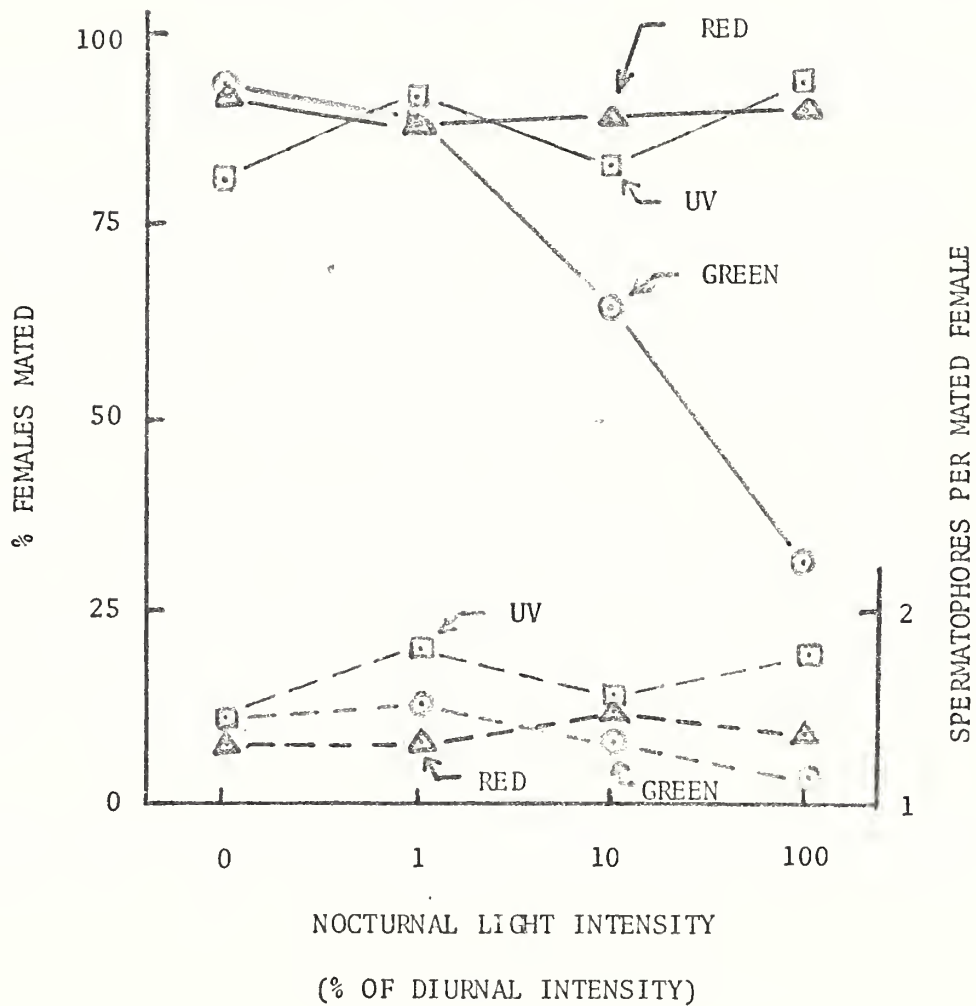


FIG. 2. MATING OF CABBAGE LOOPER MOTHS UNDER 3 COLORS OF LIGHT AND 4 INTENSITIES OF NOCTURNAL LIGHT.  
 % FEMALES MATED INDICATED BY SOLID LINES,  
 SPERMATOPHORES/MATED FEMALE BY BROKEN LINES.



## Use of Sound to Attract Male Screwworm Flies

H. R. Agee and J. C. Webb

Objective: To develop a survey system for male screwworm flies using sound produced by receptive females.

Methods: Sterilized (irradiated) laboratory reared male and female screwworm flies were used to determine premating and courtship behavior. Work thus far indicates there is much variance in behavior and vigor of laboratory reared insects. Sounds produced by laboratory reared males and females of all ages were evaluated behaviorally and analyzed electronically (frequency, pulse rate, pulse duration, sound level) to determine the most effective stimuli for attracting male flies.

Results: It was determined that female screwworm flies of the APHIS and Tex-Mex strains produced sounds by wing stridulation prior to mating that may be important in male attraction and serve as a stimulus to increase male activity and mating attempts. Different sound patterns were produced by females of the APHIS and Tex-Mex strains. Much difficulty was encountered due to the need for shipping radiation sterilized pupa from Mission, Texas. Many groups of flies displayed abnormal activity levels, reduced longevity, or arrived dead.

Plans: No further work is planned because of: (1) unavailability of wild and nonirradiated flies; (2) shipping problems, (3) lack of funds for travel to areas having wild populations of screwworm flies.



Analysis and Identification of Specialized Signalling  
Sound Produced by the Male Caribbean Fruit Fly

J. C. Webb, J. L. Sharp, D. L. Chambers, and J. C. Benner

(Continuation of report 43 73(1-6))

Objectives: To isolate and identify the specialized "signalling" sound produced by the male Caribbean fruit fly (Anastrepha suspensa) before pair formation.

Methods: Four sounds produced by fruit flies were generated by flight, aggression, premating and signalling activities. Flight sounds were recorded with the fly mounted on a flight mill; the others were recorded from caged flies. Each sound was analyzed for its frequency, waveform, and pulse content. A power spectral density curve was calculated and plotted for each of the four sounds.

Results: The acoustical properties of the flight activities show a direct relationship with fly maturity and flight ability. The wingbeat frequency was lowest for one-day-old flies (122 Hz) increased to 164 Hz at 14 days, then decreased with age. The other acoustical measurements and premating sounds were similar in their wingbeat frequency, but the waveform content was different. This indicated that these two sounds were made by different wing movements. The signalling sound was the only one produced in pulse form. This sound appears to be produced by very precise wing movements. This pulse duration varies with the age of the fly, with the shortest pulses being produced by younger flies, 6 to 8 days old, and the longest produced by flies from 10 to 15 days old. The waveform of each of these four sounds was unique, and this resulted in a specific frequency signature for each sound.

Plans: To determine if this signalling sound is an important behavioral cue and useful in manipulating the fly in the laboratory or field.



Acoustical Properties as a Means  
of Measuring Insect Quality

J. C. Webb, J. L. Sharp, D. L. Chambers, J. C. Benner,  
and B. J. Smittle<sup>1/</sup>

Objectives: To determine the changes that occur in the acoustical properties of the Caribbean fruit fly, Anastrepha suspensa (Loew) after exposure to various treatments (such as gamma irradiation, heat, diet changes, etc.).

Methods: The flies will be reared in the laboratory and after they have received the appropriate treatment the flight and signalling sounds will be recorded. Analysis of these sounds will be made to determine the differences, if any, caused by treatment effects.

Results: Flies irradiated with gamma radiation in air and in nitrogen 4-5 days before emergence were tested. The flight and signalling sounds were recorded, but the analysis is incomplete at this time.

Plans: With these data and our ability to identify behavior patterns (flight, aggression, signalling, etc.) by analyzing sound production as background information, we will evaluate the use of sound analysis as a measure of quality. Flies selected through rearing, with various nutritional or rearing practice histories, or subjected to various treatments such as irradiation or marking will be compared through sound analysis and potential detrimental effects identified.

<sup>1/</sup>Research Entomologist, Insects Affecting Man Research Laboratory,  
Gainesville, Fla.



Sound Production by Conotrachelus nenuphar: I. Morphological Description of Sound Producing Structures of Males and Females

T. Carlisle, C. Calkins, J. C. Webb, and H. R. Agee

(Continuation of report 45 73(1-6))

Objectives: The location and description of all potential stridulatory areas of plum curculios with special emphasis on sexual dimorphism.

Methods: We examined the body surface of plum curculio adults with dark phase and scanning electron microscopes to detect sites of potential sound producing structures. Detailed descriptions, measurements, drawings, and photographs of these structures and comparisons of morphological differences existing between males and females will be made.

Results: Detailed examinations of the entire body surface of male and female plum curculio adults revealed several suspect areas which may produce sound. The most likely areas involve file-like structures on the ventral surface of the posterior portion of the left elytron. These structures are sexually dimorphic. The striking mechanism on the male consists of tubercles located on the dorsal aspect of the sixth abdominal tergite. A pair of large tubercles appear to be the primary striking apparti while several paired (4-9) smaller tubercles appear to be of secondary importance. The female stridulitrum consists of teeth arranged in parallel rows that appear like file teeth at low magnification. The striking mechanism in the female does not appear to be tubercles on the abdominal tergites (although some very small ones exist), but rather a specialized area at the posterior margin of the tergite. This sound mechanism is used when the insect is distressed.

Plans: Mating experiments involving sound production will be conducted. Site of such sound production will be identified. Since different sounds are apparently produced by each sex, stridulation may be involved in sexual attraction or mating behavior. Before experiments characterizing these sounds can be completed, the sites of all sound production must be identified.



Sound Production by Conotrachelus nenuphar:  
II. Characterization and Reproduction of  
Sounds Produced in Response to Various Stimuli

J. C. Webb, C. Calkins, H. Agee, and T. Carlisle

(Continuation of report 46 73(1-6))

Objectives: To identify and characterize the different sounds produced by adult plum curculios; to ascertain the conditions necessary for the production of different types of sound.

Methods: The proper environmental conditions (temperature, light intensity, humidity, etc.) and stimuli necessary to elicit sound production will be determined. Sounds will be recorded in an anechoic chamber by using a condenser microphone. This microphone will feed data into a sound pressure level meter, then into a wide band data tape recorder where it will be stored for analysis. If necessary, the stored data will be transferred to a bin loop machine where time scaling is possible, which will allow better resolution of the analyzed data. Sounds will then be reproduced to determine reactions of beetles to various intensities and frequencies.

Results: Certain conditions were observed to stimulate adult beetles to stridulate: exposure to bright sunlight while in petri dishes; near lethal high temperatures; carbon dioxide concentrations at subanesthetical levels; and grasping of tarsi, legs or other body parts. At least 2 separate sounds were emitted when beetles were congregated in groups; their significance was not ascertained. A stridulation sound was produced by the plum curculio when they were placed under stress conditions. Preliminary tests have shown that two strokes are made during stridulation. The average pulse duration of the first stroke was 0.6 seconds, and the second was 0.9 seconds. The average number of teeth strikes was 55 and 71 for the first and second strokes, respectively. The fundamental frequency of the primary tooth stroke rate appears to vary from approximately 400 to 2000 Hz.

Plans: Continued efforts will be made to identify different sounds and the conditions necessary to produce each type. Sexes will be isolated to stimulate calling behavior. Sounds will be reproduced and played back to observe behavioral responses. If sound production is involved with mating behavior, recordings will be tested to see if mating behavior can be disrupted.



Sounds and electrical charges produced by electric-grid and black light traps that repel and/or attract insects

J. M. Stanley, H. R. Agee, and J. C. Webb

Objectives: To determine which stimuli (sounds, electromagnetic, and electrostatic) produced by electric grid and black light traps affect the behavior of insects. Determine the trapping units most attractive and least repellent to better survey and control insects.

Methods: Acoustic sense cell response of H. zea and T. ni will be determined by presenting sound stimuli produced by various trap units to determine the maximum distance that the acoustic receptors can detect the sound of arcing and non-arcing grid-traps and black light traps. The sounds will be recorded and analyzed for frequency, pulse duration, pulse rate, and sound level. The active components of the sounds will be determined physiologically and bioassayed. Similar physiological and bioassay methods will be used to evaluate the effect of electromagnetic and electrostatic charges on H. zea and T. ni.

Results: Observations and preliminary tests indicate that sounds produced by arcing and non-arcing grid traps and possibly light traps are sensed by some insects and affect their behavior.

Plans: This study will be completed within six months and will help in the design and development of more effective electric trapping equipment.



Binding of the Sex Attractant of the Almond  
and Indian Meal Moth to Antennal Proteins In vitro

S. M. Ferkovich, L. L. Sower, and R. R. Rutter

(Continuation of report 48 73(1-6))

Objective: To develop information on the mechanism of insect olfaction through studies of proteins in the antennae of the almond moth, Cadra cautella (Walker), and Indian meal moth, Plodia interpunctella (Hübner), which may function in perception of the sex attractant and related synergists and inhibitors.

Methods: Antennae were homogenized in Tris-HCl buffered sucrose (.5M), pH 7.5 and centrifuged at 20,000 g for 45 min and the supernatant analyzed on 7% polyacrylamide gels. The pheromone, (Z-E)-9,12-tetradecadien-1-ol acetate (50 µg) was preincubated with the supernatant (200 µg protein) prior to electrophoresis to determine whether pheromone binding would induce specific structural changes in certain proteins (receptors) which might be reflected as alterations in Rf values, staining intensity (sites available for stain, Coomassie blue, on pheromone-bound protein), number of subunits, etc. Pheromone degradation was monitored by extraction of the pheromone (or metabolite) from the gel and gas chromatography.

Results: In the last report we stated that when the cis, trans alcohol, an inhibitor in Cadra, was preincubated with the antennal protein from females and males and then analyzed by disc gel electrophoresis and gas chromatography, 4.7 and 11.3% of the alcohol, respectively, was bound to a slow-running high mol. wt. band. Since then we have preincubated cis-7-dodecen-1-ol, an inhibitor in the cabbage looper but not in Cadra, with male antennal protein. Only a trace amount of the cabbage looper alcohol was bound by the protein band, suggesting a high degree of binding specificity of the protein for the biologically active, cis, trans alcohol.

Plans: The alcohol binding protein(s) will be characterized, and the dissociation constant will be determined and compared with analogs of the alcohol.



Insect Antennal Esterases: Initial Purification  
and Absolute Specificity

M. S. Mayer, S. M. Ferkovich and R. R. Rutter

(Continuation of report 49 73(1-6))

Objectives: To ascertain if esterase activity in soluble antennal proteins is an integral part of the transducing process, more data are needed to determine: (1) how many esterases are present; (2) if the specificity of 1 or more of these parallels behaviorally and electrophysiologically determined specificities; and (3) if the esterases are actually located in or on the surface of the antenna and/or the receptors.

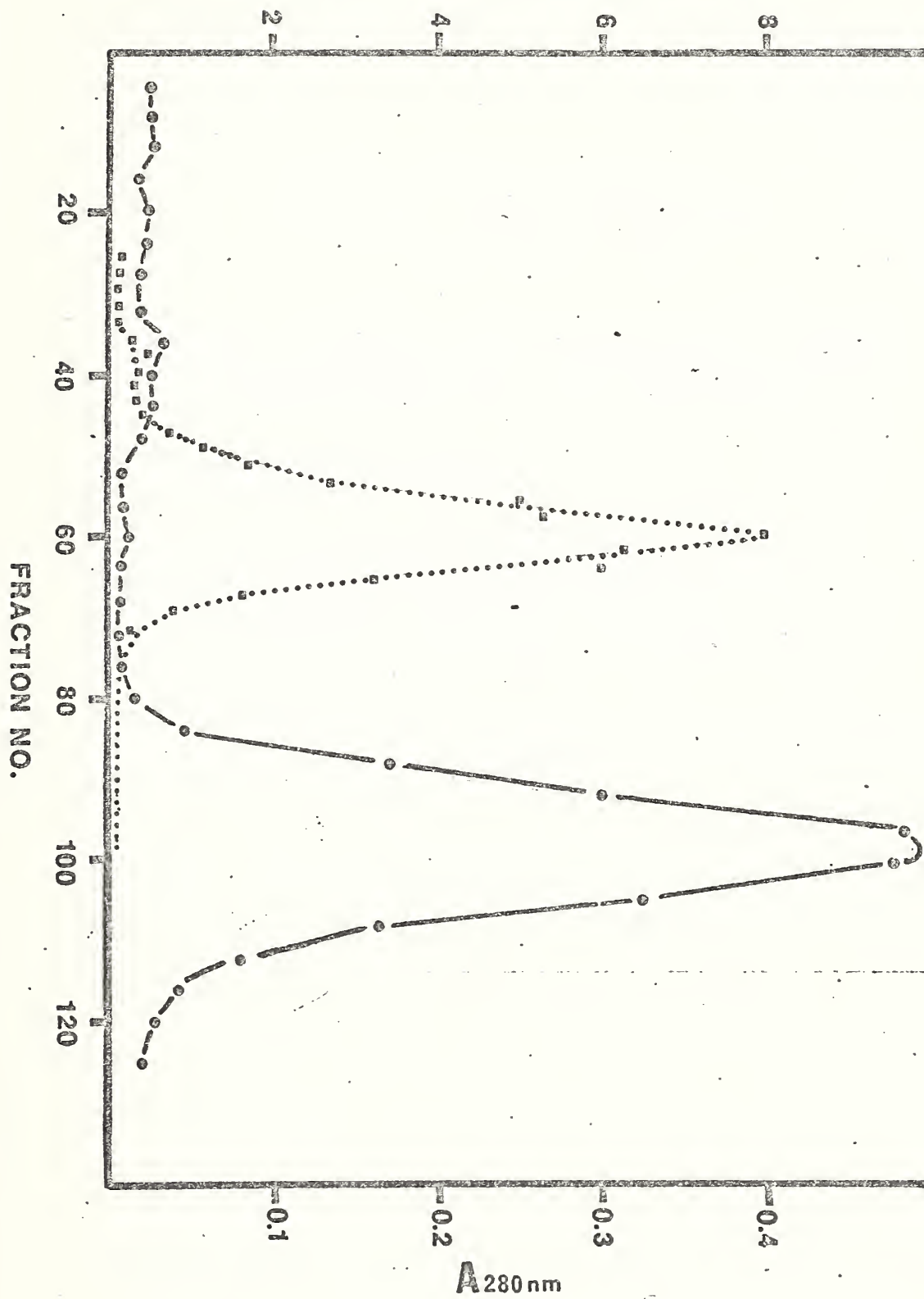
Methods: Antennal proteins were sonicated in a buffered solution containing triton X-100 before fractionation by gel permeation chromatography. To locate peaks of pheromone degradative activity, each fraction was reacted with the pheromone and analyzed by chromatography.

Results: The result of the sonication prior to gel permeation chromatography revealed only 1 peak with enzyme activity corresponding to the previously obtained peak II (Fig. 1, C.F. Fig. 1, Report 49 73(1-6)). We take this as evidence that only 1 esterase or esterase group is present in our antennal preparations.

Plans: To continue investigations into the activity of this esterase and its implications.



## PERCENT ISOMERIC ALCOHOL





Electrophysiological Investigations  
of Pheromone - Inhibitor Relationships

M. S. Mayer and J. D. James

Objectives: To determine the electrophysiological response of Plodia interpunctella (Hübner) and Cadra cautella (Walker) to their respective pheromone and behavioral inhibitors.

Methods: Electroantennograms (EAG) were obtained by standard electrophysiological techniques.

Results: Quantitative relationships were obtained between the pheromone and inhibitor for each insect. The studies demonstrated quantitative and slope differences in EAG's between the respective pheromone and inhibitor of both insect species. There was no indication in either insect that the form of the EAG itself was different in response to either pheromone or inhibitor.

Plans: To continue this investigation and attempt to correlate EAG responses to behavioral responses.



## Low Air Current Sensor

E. W. Hamilton

Objective: Electrophysiological responses of insect antennae to chemical stimuli are being studied at this laboratory. In one study, puffs of pheromone-laden air are directed at the antennae and the response noted. A means of recording the time of arrival of the air current at the antenna under study was required in order to correlate antenna response time with pheromone stimulation. The air puff sensor shown in Fig. 1 was designed for this purpose.

Description: There are 2 thermistors of the same value in the circuit. One thermistor merely serves as the ambient temperature reference so that a stable setting of the unit can be maintained. The air sensor thermistor is placed next to the antenna under study.  $V_{out}$  is connected to one input of a dual beam oscilloscope. The output from the antenna is connected to the other input of the scope. The trace on the scope then indicates when the air puff from the pheromone dispenser reaches the antenna and the response of the antenna. Antennal response time can then be determined.

The air sensor can also be used to measure or monitor very low air currents without modification. In this case,  $V_{out}$  is connected to a 50 microampere meter and the meter is calibrated relative to the air current movements being measured.



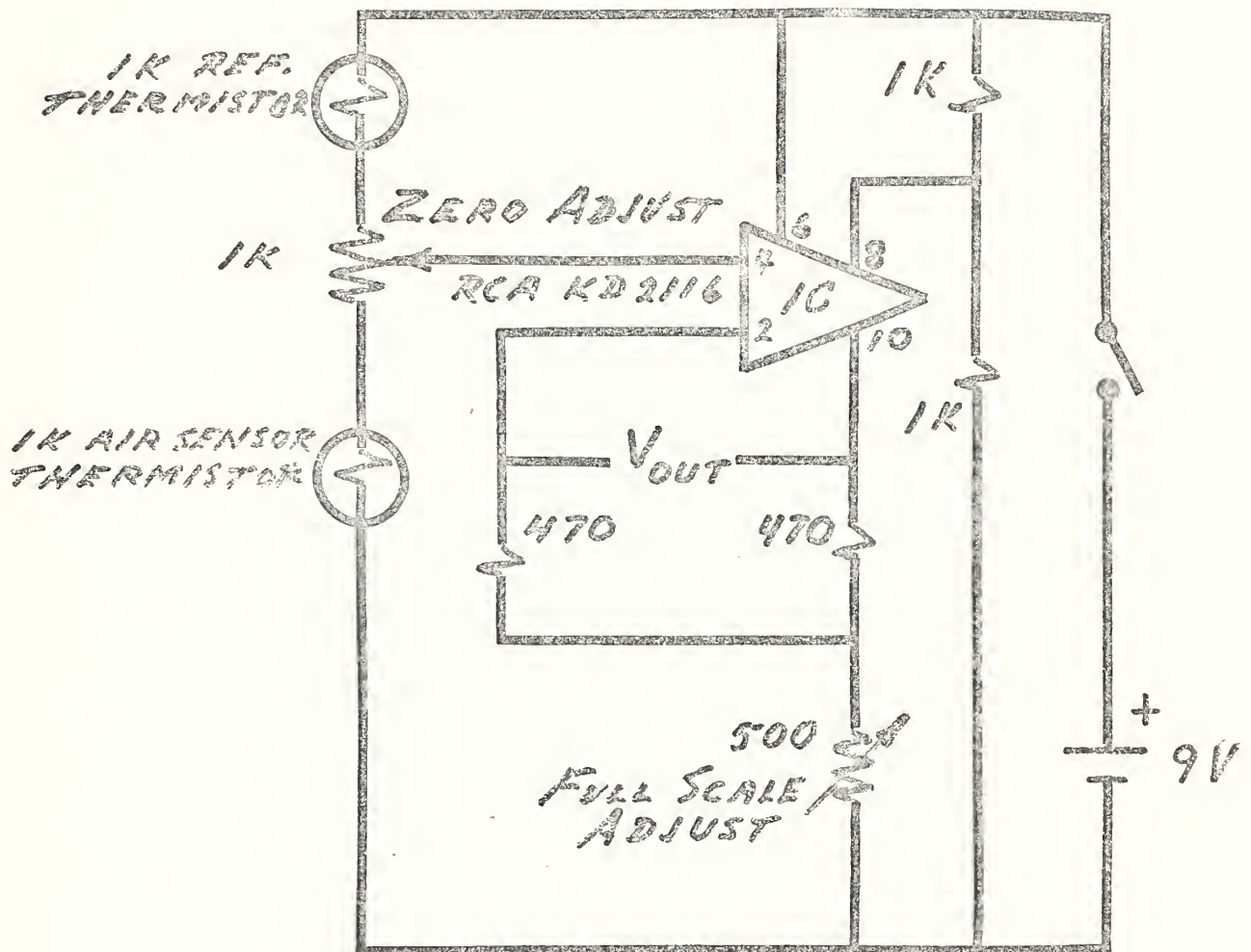


FIG. 1 AIR CURRENT SENSOR



Studies on the Infrared Emission of  
the Cabbage Looper Pheromone

P. S. Callahan

Objective: In order to understand how the emissions from insect sex attractants and host plant odors couple to the insect antennae, the infrared emission of various insect pheromones and attractive plants must be known. This project will locate and plot the emissions.

Methods: A fourier analysis spectrophotometer was modified to utilize a special 1  $\mu$ m laser source and a special chamber and mirror holder constructed so that a monomolecular layer of the pheromone could be scanned for infrared emission of frequencies. The pheromone studied was also scanned in the gas phase in a 10 cm gas cell.

Results: The cabbage looper pheromone was found to emit "bright" narrow band fluorescence in the 17  $\mu$ m and 26  $\mu$ m IR region. Both IR lines match the length of two groups of trichodea sensilla on the cabbage looper antenna. The 17  $\mu$ m region line emits log periodically. The spacings of the sensilla on the antennae are log periodic (Fig. 1&2).

Plans: Further work is in progress in which the molecule is irradiated with various wavelengths of IR, in order to find and plot a 3rd frequency believed to be in the 45 to 55  $\mu$ m region of the spectrum. There is also a group of trichodea sensilla within that range on the cabbage looper antennae.



log periodic fluorescence (open phase)

600 cm<sup>-1</sup>

1500 cm<sup>-1</sup>

1600 cm<sup>-1</sup>



total distance - all light out

2 1500 cm<sup>-1</sup> 1600 cm<sup>-1</sup> 1700 cm<sup>-1</sup>

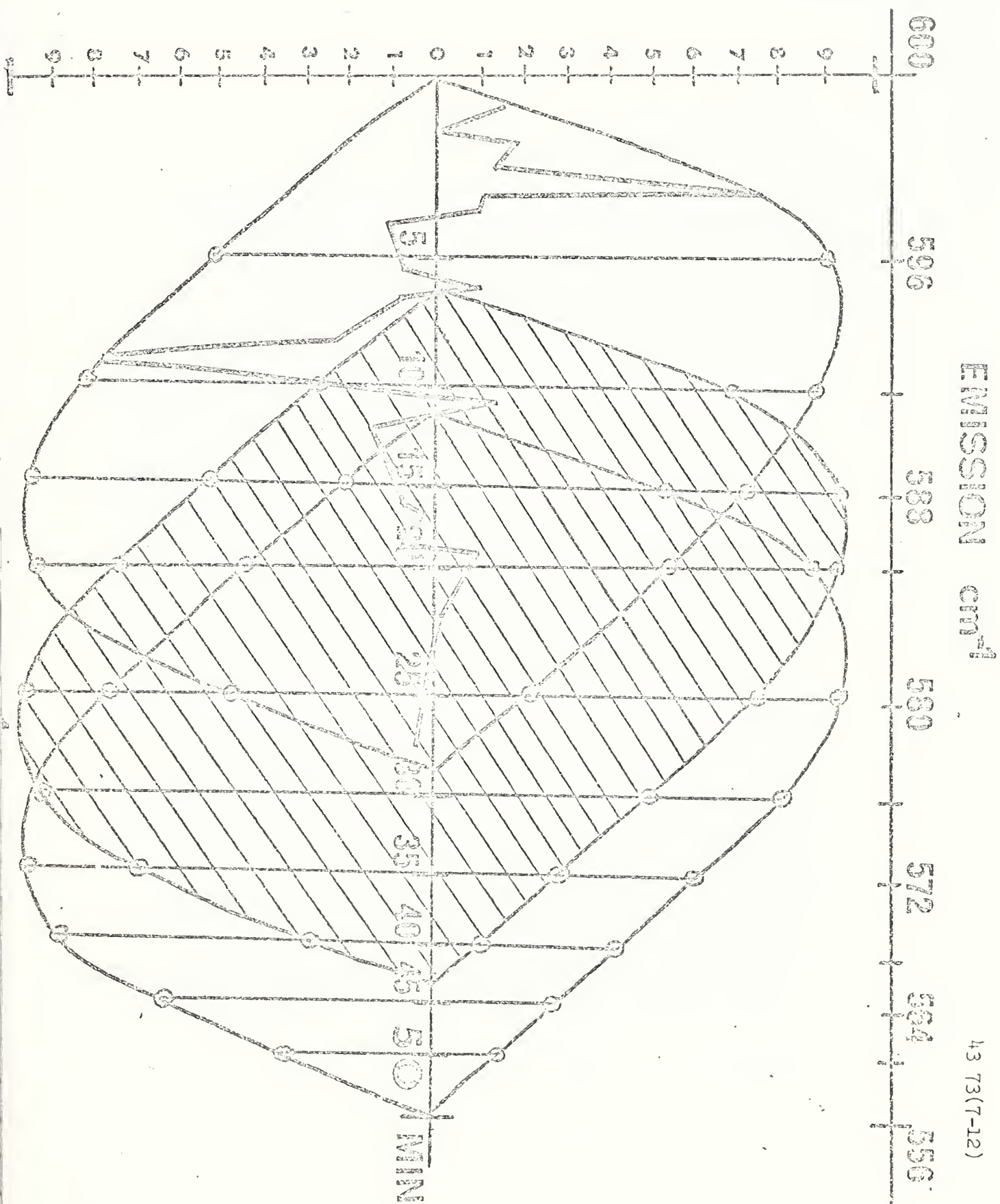
5 1800 cm<sup>-1</sup> 1900 cm<sup>-1</sup> 2000 cm<sup>-1</sup>

500 cm<sup>-1</sup>

1500 cm<sup>-1</sup>

Fig. 1. Log periodic 17  $\mu$ m region IR emission (fluorescence) from the cabbage looper pheromone.





43 73(7-12)

Fig. 2. One emission line (up)  $598 \text{ cm}^{-1}$  and absorption line (down)  $590 \text{ cm}^{-1}$  in the  $17 \text{ }\mu\text{m}$  region. The emission (fluorescence) moves log periodically by frequency and with time from  $598 \text{ cm}^{-1}$  to  $556 \text{ }\mu\text{m}^{-1}$  ( $16.7$  to  $18.0 \text{ }\mu\text{m}$ ).



Effect of Increasing Levels of Gamma Irradiation  
on the Histological Structure of the Compound Eye  
on the Caribbean Fruit Fly

H. R. Agee, J. L. Sharp, J. C. Webb,  
B. J. Smittle<sup>1/</sup> and M. L. Park

Objective: To determine the effect of different levels of gamma irradiation on the structure of the compound eye of the Caribbean fruit fly.

Methods: Histological sections of stained preparations of the compound eye of the Caribbean fruit fly irradiated with 5 and 10 kR at different ages in air and nitrogen will be examined with the light microscope and these structures compared to those of nonirradiated flies of both sexes.

Results: Preliminary results indicate that the pseudocones of the compound eyes of the females receiving 10 kR were abnormal and may affect normal vision.

Plans: Examination of other treatments will continue and electroretinograms of normal and treated flies will be compared.

<sup>1/</sup> Research Entomologist, Insects Affecting Man Research Laboratory, Gainesville, Fla.

(SI-1) 1/2 1/2

Effect of Wind Speed and Direction on the  
Approach of Soybean Loopers to a Pheromone Source

W. K. Turner and E. W. Hamilton

Objective: To determine the influence of wind speed and direction on the number of soybean loopers, Pseudoplusia includens (Walker), attracted, and the direction of their approach, to a pheromone source.

Methods: Six 3 X 3 ft. electric grid panels were erected in an hexagonal pattern in an open field. Surrounding fields contained plots of soybeans. The grids were instrumented so that the impact of an insect on a particular grid was recorded with an event recorder. Insects killed by the grids were counted daily. Wind speed and wind direction were recorded with a Bendix Model 141-2 wind recorder. The traps were baited by suspending a plastic vial of soybean looper pheromone (Z-7-dodecen-1-ol acetate) in the center of the trap. Data were collected during September of 1973.

Results: During a 3-week period, approximately 5,000 soybean loopers, and an equal number of other insects, mostly velvetbean caterpillars, Anticarsia gemmatilis Hübner, were killed by the grids. During the same period, approximately 17,000 insect impact events were recorded. (The difference in the figures is explained by the small insects which actuated the event recorder, but were not included in the data). There was a strong correlation between wind direction and direction of approach of the insects toward the trap; a large percentage struck the leeward grids. This was true for soybean loopers, and all other insects. There was an increase in the nightly kill of soybean loopers with increasing wind speeds, up to the maximum wind which gusted to about 5 mph. The average wind speed was about 1 mph throughout the night. However, total number of insects striking the grids was only weakly correlated with wind speed, if at all, except that with wind speeds less than the measuring capability of the wind recording equipment, fewer insects were killed.

Plans: Additional data will be collected during the coming season.



The Flight Ability of Lesser Peachtree Borers  
in the Laboratory

J. L. Sharp, J. R. McLaughlin, and D. R. Bennett

Objectives: To determine the flight ability of lesser peachtree borers, Synanthedon pictipes.

Methods: Lesser peachtree borer males, S. pictipes, from the laboratory colony were fastened to the flight mill system when they were 1 day old and allowed to fly for ca. 60 min.

Results: Preliminary results are shown in Table 1.

Plans: Sex, age, irradiation, and temperature effects on flight will be studied. their movement in the field will be evaluated. Also, their wingbeat frequencies will be investigated stroboscopically.

Table 1. Mean flight data of S. pictipes.

Flight categories	1 day
Distance (m)	555.7
$\bar{X}$ flight velocity (m/sec)	0.57
Peak velocity (m/sec)	0.70
% time flying	22.2
$\bar{X}$ no. flights/hr	16



The Flight Ability of Trichoplusia ni in the Laboratory

J. L. Sharp, D. R. Bennett, J. R. McLaughlin, and T. Antonio

Objectives: To determine the flight ability of T. ni in the laboratory.

Methods: Cabbage loopers from our stock laboratory colony were fastened to the flight mill system and allowed to fly for ca. 60 min. Males and females were flown each day when they were 1, 2, 3, 4, and 5 days old.

Results: Preliminary results are shown in Table 1.

Plans: The effects of temperature on flight will be investigated as well as sexual condition and ability to fly over a 24-hr period. Wild T. ni adults will be compared to our laboratory colony on the mill and in field release studies. Similar comparisons will be conducted with the beet and fall armyworms.

Table 1. Mean flight data of T. ni.

Categories	Age (♂-♀)				
	1 day	2 day	3 day	4 day	5 day
Distance (m)	1671-2944	2320-1998	2298-2281	1812-1999	496-1893
$\bar{X}$ flight velocity (m/sec)	1.00-1.14	1.24-1.07	1.07-0.96	0.59-1.15	0.92-1.11
Peak velocity (m/sec)	1.03-1.36	1.33-1.11	1.10-1.15	0.64-1.30	1.02-1.18
% time flying	69.5-61.1	20.1-54.6	50.0-62.4	22.9-81.9	21.0-63.4
$\bar{X}$ no. flights/hr	1.8-2.2	4.0-2.6	4.6-5.3	13.8-5.6	8.6-4.4

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The Effects of Gamma Irradiation, Age, and Sex  
on the Wingbeat Frequency of Anastrepha suspensa

J. L. Sharp, D. R. Bennett, and B. J. Smittle<sup>1/</sup>

Objectives: To determine the effects of <sup>60</sup>Co, age, and sex on the wingbeat frequency of A. suspensa.

Methods: Laboratory-reared A. suspensa pupae from Homestead, Fla. were gamma irradiated in Gainesville at levels of 2.5, 5, 10, and 20 kiloröntgens (kR) 2 days before adult eclosion at a dose rate of ca. 1 kR/min. A separate batch was not irradiated and used as controls. Wingbeat readings were taken periodically from 1 to 25 days using a Strobotac<sup>®</sup>.

Results: The frequencies for 1-day-old sexes were consistently lower than others, regardless of the treatments. The heavier females exhibited higher frequencies than males in 4 instances. The wingbeat of 20 kR-irradiated males at ages of 4, 7, and 25 days was significantly reduced compared to flies irradiated at doses of 2.5, 5, and 10 kR and nonirradiated flies. No wingbeat data were collected from females older than 7 days due to excessive mortalities from 20 kR. The adverse effect to wingbeat frequency from 20 kR was not as severe as it was for longevity.

Plans: This aspect of the research has been completed; however, further work is contemplated using stroboscopic analysis to determine the quality of wild and laboratory-reared insects exposed to various components of the mass production and treatment process.

<sup>1/</sup>Research Entomologist, Insects Affecting Man Research Laboratory, Gainesville, Fla.



Attraction of Stable Flies to Heated Objects

W. K. Turner, R. S. Patterson<sup>1/</sup> and D. W. Meifert<sup>1/</sup>

Objective: To explore the possibility of using a heat source as an attractant in a stable fly trap and to determine the infrared sensing capabilities of the fly.

Methods: Populations of starved stable flies, Stomoxys calcitrans (Linnaeus), were placed in cages made of fiberglass screen. Heated objects of various sizes, materials and temperatures were introduced into the cage, and flies collecting on the objects were counted.

Results: Stable flies were attracted to objects heated to 80°F or above, with the laboratory ambient temperature at 70°F. Maximum attraction and stimulation of activity resulted with small sources, such as a soldering iron tip, heated to about 110°F. Temperatures above 120°F caused attraction and subsequent repulsion.

Plans: Work will continue to determine optimum size, color and temperature of attractive objects. Traps will be constructed and tested.

<sup>1/</sup>Research Entomologists, Insects Affecting Man Research Laboratory, Gainesville, Fla.



Role of Dispersal and Life History Characteristics in the  
Population Dynamics of the Almond Moth, Cadra cautella, in a  
Citrus Pulp Warehouse

D. W. Hagstrum and Jane Sharp

(Continuation of report 68 73(1-6))

Objective: This study seeks to identify and describe aspects of the insect's behavior and life history that might be used to improve current methods of control or develop new ones.

Methods: The larval population of the almond moth in a citrus pulp warehouse was sampled weekly using artificial pupation sites. Insects marked with body or eye color mutations were released and recaptured to evaluate the relationship between population density and trap efficiency. Moths were reared at 8L:16D, 10L:14D, 12L:12D, 14L:10D, 16L:8D and in darkness to determine the role of photoperiod in the initiation of diapause. Small bags of citrus pulp were interspersed among larger bags and will be recovered later to determine the age structure of the immature population.

Results: Studies have shown that the almond moth completes 4 generations within the first 31 weeks that citrus pulp is stored. Throughout the period of storage, moths diapaused in the citrus pulp warehouse and the incidence of diapause among mature larvae increased. Based upon the interval between peaks, the generation time was 6-8 weeks. The percentage of marked larvae that were recovered was unaffected by the number released or the density of mature larvae in the release area. Results of studies on diapause and age structure are not yet available.

Plans: Studies on diapause and population dynamics will be continued.

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Dispersal of the Red Flour Beetle,  
Tribolium castaneum Within Flour

D. W. Hagstrum

(Continuation of report 70 73(1-6))

Objectives: Knowledge of the behavior of insects infesting a commodity is a prerequisite to the development of better methods of detection. New information on the insect's behavior might also be exploited for purposes of control. The dispersal of insects within the commodity is particularly important.

Methods: The movements of Tribolium castaneum in a 10 cm high X 0.64 cm diam. column of flour and a 10 cm wide X 15 cm high X 0.64 cm deep column of flour were recorded by autoradiography. Frequency distributions for the angles, distances and rates at which T. castaneum moves through flour were used to simulate the dispersal of beetles in a planar space 200 cm in diam. The program also has the ability to simulate movement in 3 dimensions.

Results: A computer program has been written and debugged, but results are not yet available.

Plans: Simulation studies will be continued. Additional aspects of the beetles behavior, such as encounters between beetles, will be considered. The potential that the model and program might have in studying attributes of dispersal common to many species of insects will be investigated. Eventually, we hope to relate dispersal pattern to sampling strategy.



Role of Constant and Fluctuating Temperatures in  
Determining the Behavior and Development Time of  
Stored-Product Insects

D. W. Hagstrum

(Continuation of report 69 73(1-6))

Objectives: Differences in an insect's response to constant and fluctuating temperatures are often important to the prediction and manipulation of behavior, life history or physiology. This study seeks to identify and quantitate these differences.

Methods: The development times of the red flour beetle, Tribolium castaneum and almond moth, Cadra cautella, were determined for 8 populations of 50 insects that were exposed to constant temperature. Eight populations of similar numbers were exposed to sinusoidal temperature cycles with 10°C ranges. Mean temperatures of 22.5, 25, and 35°C were compared.

Results: Only the results for T. castaneum at 35°C are available and these are the same as those obtained with previous replicates.

Plans: Studies will be expanded to include other temperatures. Further work will be initiated to determine the reason for differences in the response of these insects to the two environments.



Development of Genetically Marked Strains of the  
Plum Curculio, Conotrachelus nenuphar (Herbst)

M. Huettel, C. Calkins, A. Hill, and J. Young

(Continuation of report 74 73(1-6))

Objective: Strains of the plum curculio genetically marked by rare isoenzyme alleles are being developed for use in field studies of the biology and ecology of this insect.

Methods: Twenty-five single pairs were established on, and their progeny reared from, green thinning apples. Subsequently, a scheme of inbreeding and sib selection, monitored by starch gel electrophoresis, was used to breed lines homozygous for certain rare isoenzyme alleles. These inbred lines were then pooled to form outbred marked strains.

Results: Homozygous lines were produced by inbreeding in the F<sub>3</sub> and F<sub>4</sub> generations. From these lines, 4 strains have been established. Each strain is homozygous for a rare allele at one of 4 enzyme loci.

Plans: Wild plum thickets at the Tall Timbers Research Station, Leon Co., Fla., will be mapped during the spring bloom period. Suitable sites will be selected for release of one or more of these marked strains. Studies of the incorporation of laboratory reared genetic material into wild populations will be made. Follow-up studies will also be made of movements between plum thickets. Similar studies of movement between cultivated fruit hosts are planned in cooperation with the Southeastern Fruit and Tree Nut Research Laboratory at Byron, Ga.



Genetic Variation Between Populations of the  
Plum Curculio, Conotrachelus nenuphar (Herbst)

M. Huettel, C. Calkins and J. Young

Objectives: A study has been undertaken to investigate dispersal patterns and the possible existence of host races in the plum curculio.

Methods: Techniques for the visualization of about 16 enzyme loci by means of starch gel electrophoresis have been developed. In a preliminary survey, samples of larvae were collected from (1) wild plums, Gainesville, Fla.; (2) nectarines, Madison, Fla.; (3) peaches, Monticello, Fla.; (4) cultivated plums, Byron, Ga.; and (5) a laboratory strain reared on apples. Allele frequencies at 5 enzyme loci were determined by electrophoresis.

Results: The results of this preliminary study are given in Table 1. Lactate dehydrogenase (LDH) is monomorphic, i.e., all populations are fixed for the same allele. Phosphoglucumutase (PGM) locus has 2 segregating alleles in 4 of the 5 populations. Isocitrate dehydrogenase (IDH), glutamic-oxaloacetic transaminase (GOT) and alcohol dehydrogenase (ADH) each have multiple alleles. While variations between hosts and between localities are confounded in these data, it appears that there is adequate variability between populations to warrant further investigation.

Plans: An extension of this study to more loci over a broader geographic range, comparing where possible populations from sympatric host types is planned. The extent of isolation between the uni- and multivoltine strains of this pest will also be examined using these techniques.



Table 1--Allozyme allele frequencies in plum curculio populations

Locus	Locality	Alleles								n
		1	2	3	4	5	6	7	8	
IDH	Gainesville	1.0000								37
	Madison	1.0000								41
	Monticello	1.0000								31
	Byron	1.0000								30
	Lab Strain	1.0000								24
PGI	Gainesville	1.0000								44
	Madison	.9694	.0306							50
	Monticello	.6270	.3730							53
	Byron	.8791	.1209							51
	Lab Strain	.8750	.1250							24
IDH	Gainesville		1.0000							53
	Madison	.0545	.9409	.0045						110
	Monticello	.0267	.9400	.0333						75
	Byron	.0168	.9382	.0449						89
	Lab Strain	.0056	.7584	.2360						24
GOT	Gainesville		.9340		.0189					53
	Madison	.0472	1.0000							79
	Monticello		1.0000							73
	Byron	.0197	.9210	.0329	.0263					76
	Lab Strain		1.0000							24
ADH	Gainesville	.0732		.1707	.1629	.0122	.5510			41
	Madison	.0476	.0048	.2619	.0143	.0095	.6428	.0190		105
	Monticello	.2581		.2500	.0161		.4758			62
	Byron	.0329	.0132	.2434	.0066		.6974		.0066	76
	Lab Strain	.0417		.1667			.7917			24



Hibernation Studies of the Plum Curculio,  
Conotrachelus nenuphar (Herbst)

C. O. Calkins and M. McKoy

Objectives: To determine the overwintering mortality and the date of active emergence from hibernation quarters for plum curculios in Florida.

Methods: Plum curculios, reared under semi-outdoor conditions for 2 generations, were placed in 5-gallon screened containers at 3 locations in Florida: at Quincy, at the Tall Timbers Research Station, and at Gainesville. Cans were placed both in well-protected wooded areas and in more open orchard situations at each location. Weekly observations of beetle activity at Gainesville are being made throughout the winter. Mortality will be determined at all locations during January and February.

Plans: Mortality and emergent dates will be determined for several years in conjunction with environmental measurements to determine the effect of winter weather on overwintering success and spring emergence.



Seasonal Reproductive Capabilities of Selected  
House Fly Parasites

F. C. Tingle and E. R. Mitchell

Objectives: Determine the reproductive capabilities of 2 species of house fly parasites, Mucidifurax raptor Girault and Sanders and Spalangia endius Walker, at summer, winter, and spring/fall conditions occurring in the Gainesville, Florida area. The effect of host density and exposure time to host are also being observed. A wild culture of M. raptor is being compared to a culture obtained commercially from California.

Methods: Cultures of M. raptor and S. endius are being maintained in the laboratory for testing in environmental chambers adjusted for the seasonal conditions. A wild culture of M. raptor was obtained by exposing lab-reared house fly pupae contained in plastic pans to insect populations at poultry farms where no parasite releases had been made.

Mated female parasites are exposed singly to either 20 or 40 house fly pupae for 24 or 48 hours at the selected seasonal condition. The house fly pupae are then held for emergence of adult insects.

Results: Some treatments are still in progress; therefore, complete data will be available for the next report.

Plans: Tests will be completed and evaluated.



Parasites of the House Fly, Little House Fly  
and Soldier Fly in Poultry Houses

E. R. Mitchell, W. W. Copeland, C. A. Morgan, and F. C. Tingle

(Continuation of report 77 73(1-6))

Objective: Determine the species of parasites associated with house flies (Musca domestica), little house flies (Fania sp.) and soldier flies (Hermetia illucens) breeding in poultry manure.

Methods: Samples of poultry manure and sand (1/2 liter of each) were collected at weekly intervals beginning in April and continuing through the present from several commercial egg farms near Lake Butler, Florida. House fly, little house fly, and soldier fly pupae were collected and held in the laboratory for emergence of parasites.

Results: Six parasitic species in 4 families emerged from the puparia collected (Table 1). Of the species found, M. raptor, S. endius, and S. cameroni are most numerous. Aphacreta muesebecki was previously known only from Mississippi.

The soldier fly parasite, Trichopria, is as yet undescribed. An undescribed species of Trichopria found in southern California is reported to be a pupal parasite of the house fly, little house fly, and stable fly. In laboratory studies, we were successful in inducing Trichopria n. species to attack soldier fly pupae; however, it did not attack soldier fly larvae or larvae and pupae of the house fly. It therefore appears reasonable to conclude the Trichopria reported here is not a parasite of the house fly, and it apparently is a different species than that reported from California.

Plans: The survey has been terminated due to travel restrictions.

Publications: See App. 2 NTE: E. R. Mitchell, W. W. Copeland, C. A. Morgan, and F. C. Tingle



Table 1--Parasitic Hymenoptera reared from fly pupae, Lake Butler, Fla. 1973.

Parasitic species	Host species		
	House fly	Little house fly	Soldier fly
Pteromalidae			
<u>Mucidifurax raptor</u>	X	X	
Braconidae			
<u>Aphaereta muesebecki</u>	X	X	
Spalangidae			
<u>Spalangia cameroni</u>	X	X	
<u>Spalangia endius</u>	X	X	
<u>Spalangia nigra</u>	X		
Diapriidae			
<u>Trichopria</u> n. sp.			X



Hermetia illucens (L.): Rearing and Parasites

F. C. Tingle and E. R. Mitchell

(Continuation of report 78 73(1-6))

Objectives: Mating and oviposition by the soldier fly, Hermetia illucens, in large field cages was reported previously. Attempts then were made to rear H. illucens through its complete life cycle in the greenhouse. Rearing of the soldier fly parasite, Trichopria n. sp., also was attempted.

Methods: Soldier fly pupae were collected in the field and adults were allowed to emerge in cages held in the greenhouse. Eggs were collected and placed on CSMA larval fly medium for development.

Results: Under greenhouse conditions (ca. 85°F), adult soldier flies emerged in 38 days. However, there was very little mating and oviposition by insects held under these conditions.

The life cycle of the soldier fly parasite, Trichopria n. sp., averaged 26 days when held at 80°F in an environmental chamber. We were unable to get this parasite to reproduce on house fly larvae or pupae or soldier fly larvae; however, it did reproduce on soldier fly pupae.

Plans: There are no present plans to continue this research.



Population Dynamics of the Velvetbean Caterpillar,  
Anticarsia gemmatilis

N. C. Leppla, G. D. Butler<sup>1/</sup>, and R. H. Guy

Objectives: To determine the duration of each stage of the life cycle of this species following development at 4 constant temperatures, and to generate a population dynamics model based on the "WATBUG" program.

Methods: Survival and development of velvetbean caterpillar, Anticarsia gemmatilis Hübner, larvae will be determined by placing 1600 neonate larvae on diet in 1-oz plastic cups (1 larva/cup). These cups will be maintained in incubators at 15.6, 21.1, 26.7, or 32.2°C in a saturated atmosphere with a light:dark 14:10 photoperiod. Date of infestation, date of pupation, duration of the pupal stage, sex of pupae, and percentage survival will be recorded. On adult day 1, 50 male and 50 female moths from each regime will be transferred to wire oviposition cages and maintained in their respective larval environments. Daily mortality, sex of the dead moths, number of spermatophores transferred/mated female, and the number and percentage hatch of eggs will be noted for each population.

Results: Larvae mature at each of these temperatures; however, moths are probably less tolerant. The influence of constant temperature on this species will be determined by recording; (1) mean generation time by stage, (2) survival of each stage, (3) mating frequency, (4) male and female emergence curves, (5) rate of oviposition, and (6) percentage of egg hatch for moths maintained under each regime.

Plans: The procedures will be replicated 3 times and the results will be correlated with mean annual temperatures from Homestead, Orlando, and Quincy, Florida.

<sup>1/</sup> Western Cotton Research Laboratory, 4135 E. Broadway, Phoenix, Arizona



## Computer Programs for the Analysis of Entomological Data

T. R. Asuley

Objectives: To set up a series of computer programs written in Fortran which have general application to the analysis of entomological data. Each program will have a set of instructions describing the experimental design and types of data it will analyze, how the data are punched on the computer cards, and an example of the output. This will allow a prospective user to evaluate the program's potential to aid him in his data analysis and will also permit those not familiar with computer techniques to use these programs.

Results: Three computer programs are planned for this series. The first has been completed and a preliminary manuscript prepared on its application. This program analyzes parasitoid-host or predator-prey studies conducted in open fields, field cages, greenhouses, or in the laboratory. Each replicate and treatment within an experiment is analyzed in such a manner that a mathematical picture is produced of how the natural enemy dispersed, destroyed hosts, and reproduced from the point of release. The second program is about 1/2 completed. Its primary function is to analyze fecundity and longevity studies. This second program now gives an arithmetical analysis of the data as well as a set of descriptive statistics for each replicate and treatment in an experiment. The third program is specific in that it is used to analyze flight mill studies and other behavioral events.

Plans: To the fecundity and longevity program will be added Cal-Comp plotter routines, life table output, and a set of statistical tests. The computer program analyzing flight mill studies which was written and worked on by other individuals will undergo major revision and further debugging in order to correct a recording error, remove several logical errors, and increase the amount of meaningful output.



Comparison of Electric Grid Trap Sizes and  
Electrode Spacing for Hornworms

J. M. Stanley, J. C. Webb, L. R. Mitchell and C. Masuda

(Continuation of report 63 73(1-6))

Objectives: Cylindrical electric grid traps are very effective in the evaluation of pheromones of some insects. Preliminary to studies of the tobacco hornworm sex pheromone, an effort has been made to use the grids to study the attraction of males to virgin female moths and to determine the most satisfactory size grid and electrode spacing for these large moths. Some observations have been made that these moths are able to move vertically through the cylinder formed by the grid without being caught; therefore, we also need to determine the frequency of this activity.

Methods: Five cylindrical grid designs were used: two were 12 in. in diameter with the electrode spacing on one 1/4 in. and the other 1/2 in.; one was 18 in. in diameter with 1/2 in. electrode spacing; and two were 24 in. in diameter with the electrode spacing on one 1/4 in. and on the other 1/2 in. In order to determine the movement through the cylinder, two grids of each size were used, one covered with hardware cloth to prevent the passage of moths and the other uncovered. All the grids were elevated in the funnels so that their geometric centers had the same angular relationship with the edges of the trap funnels. Each grid was operated with a 4 ma 120 volt current limiting transformer. They were placed on a line perpendicular to the direction of the prevailing wind adjacent to a field on which tobacco was grown in 1972. Grids were baited with three virgin female moths every 2 days and traps were rotated daily.

Results: Moth collections during 3 complete rotations of the grids were very erratic and an analysis of the data showed no significant difference among them.

Plans: It appears that the 12' diameter grid without a wire cover on the top is a satisfactory unit for use. This size will be used in evaluating chemical insect attractants and measuring certain specific insect populations until a more effective means is developed.



Battery Operated Survey Light Trap

J. M. Stanley, J. S. Smith<sup>1/</sup>, and L. E. Campbell<sup>2/</sup>

Objective: To design a black light portable insect trap to be used by APHIS personnel and others conducting insect surveys.

Method: A plan for an A.C. operated electric survey trap has been prepared following a request from APHIS. It is desirable for the unit to be interchanged between an A.C. and D.C. power supply for wider use. Inverter equipment for 15-watt fluorescent lamp operation from an automotive type storage battery that will provide efficient and effective operation is needed. Commercially available inverters will be investigated. Consideration will be given to designing an appropriate unit.

Results: A new commercially available inverter ballast with photoelectric control was obtained. Preliminary tests indicate that a fully charged heavy duty storage battery will supply energy for operating the lamp 5 nights. This is an improvement over inverters previously used.

Plans: Further testing on efficiency and effectiveness will be conducted.

<sup>1/</sup>Agricultural Engineer, Southeastern Fruit and Tree Nut Laboratory, ARS, Byron, Ga.

<sup>2/</sup>Agricultural Engineer, Physical Control Laboratory, Environmental Quality Institute, ARC-East, Beltsville, Md.



## Light Switch for Battery Powered Insect Traps

E. W. Hamilton

Objective: Battery-powered light traps and electrocutor traps are generally operated during night hours. In order to conserve battery energy, no power should be consumed when operation of the traps is not desired. A simple light switch has been designed to perform this duty.

Description: The light switch shown in Fig. 1 has been designed to turn on power at dusk and off at dawn. Negligible power is used when the switch is off since current drain from the battery is limited by several hundred thousand ohms of resistance. When light in the evening reaches a level low enough to increase the resistance of the phototransistor to a value greater than that of the fixed and adjustable resistance, transistor TR is turned on and the relay is activated. Power is supplied to the equipment via the relay contacts. A simple modification (Fig. 2) converts the light switch to supply power during the daylight hours. The relay used should have a coil rating of 6 volts and about 10 ma. Current drawn through the relay contacts is limited by the load rating of the contact points. If higher current carrying capacity is required, the relay contacts can be used to turn on a power relay or a power transistor with an adequate load rating.



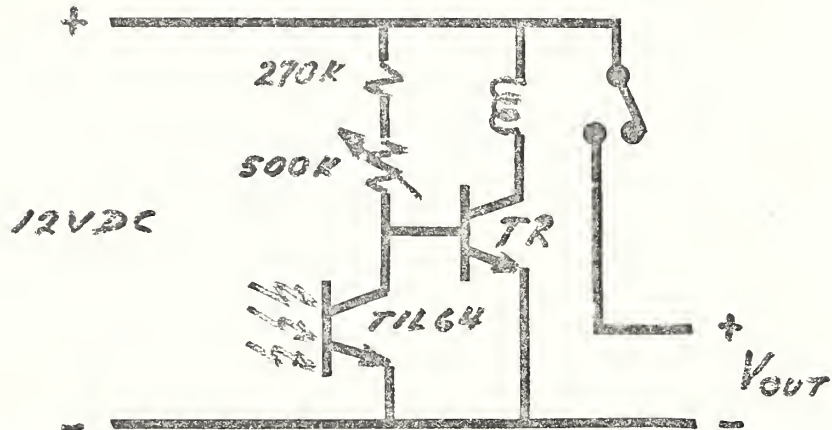


FIG. 1 LIGHT SWITCH - NITE TURN-ON

TR = HEP 50007 OR EQUAL

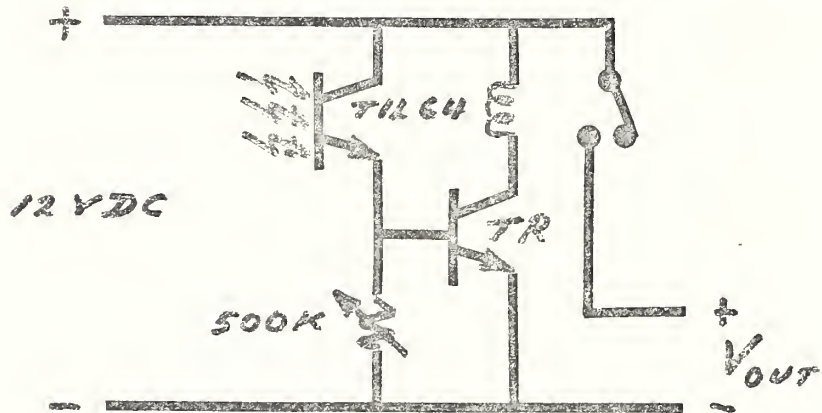


FIG. 2 LIGHT SWITCH - DAY TURN-ON



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- \*Childs, James and Thelma Carlisle. New aspects of blight disease of citrus.
- Leppla, Norman C., Robert E. Doolittle and James D. Solomon. Culturing Lepidoptera: The carpenterworm, Prionoxystus robiniae.
- \*Nguyen Ru, W. H. Whitcomb and T. Carlisle. Recent investigation on the life history of Crysopa lanata (Neuroptera: Crysopidae).
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- \*Ashley, Tom R. and D. Gonzalez. Computer program for analyzing parasite-host or predator-prey relationships.
- \*Calkins, C. O. V. M. Kirk and Betty Dupraz. Life history and mass rearing of Embaphion muricatum (Say) (Coleoptera: Tenebrionidae).
- Carlyle, S. L., W. J. Pons, N. C. Leppla and E. R. Mitchell. Adaptation of a rodent rearing container for culturing Lepidoptera in the laboratory.
- Hagstrum, David W. and Jane E. Sharp. Population dynamics of Cadra cautella (Walker) in citrus pulp and confirmation of diapause.
- \*Leppla, Norman C. and Mont A. Cazier. Habits of the hypertrophic blister beetle, Cysteodemus armatus.
- Mayer, M. S. An inquiry into attraction and inhibition of Trichoplusia ni.

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\*Indicates manuscripts prepared here, credited to another organization.



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PUBLICATIONS LIST

February 1969 -

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